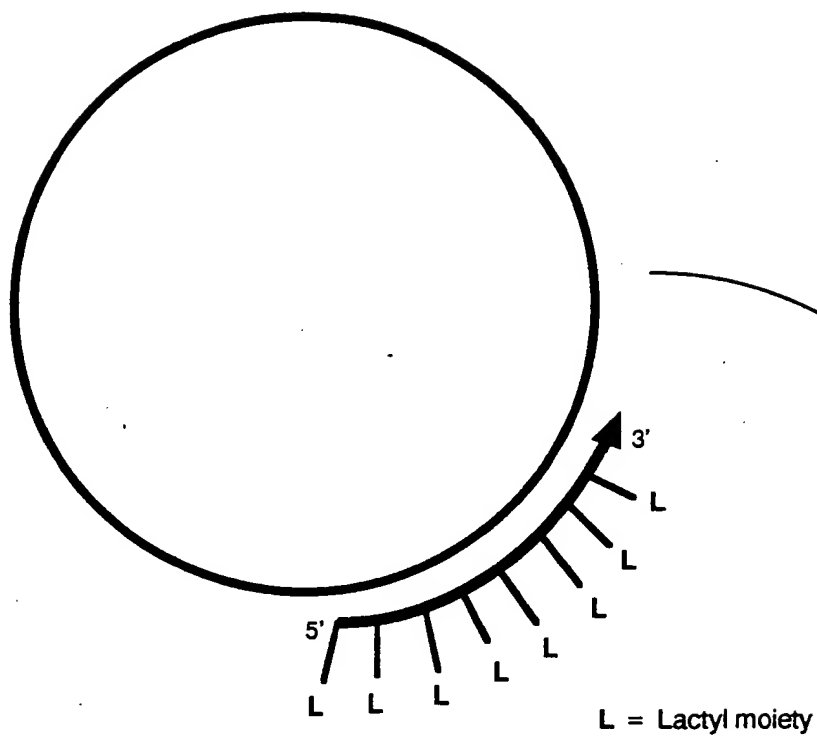
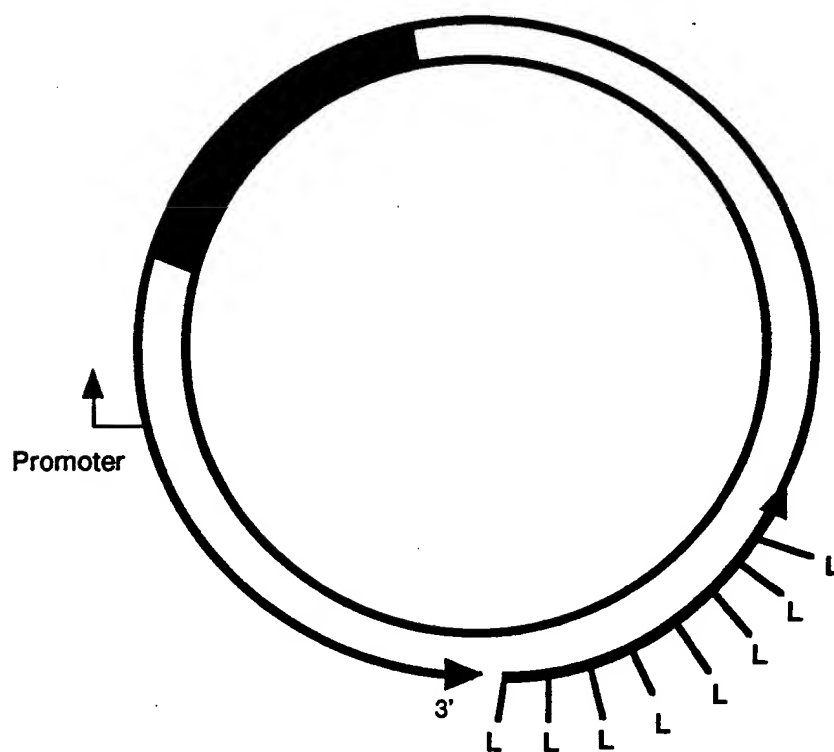


(a)



(b)

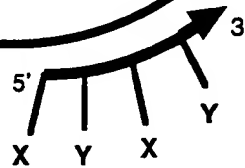
**Figure I**

Attachment of Ligands Through Primer Region

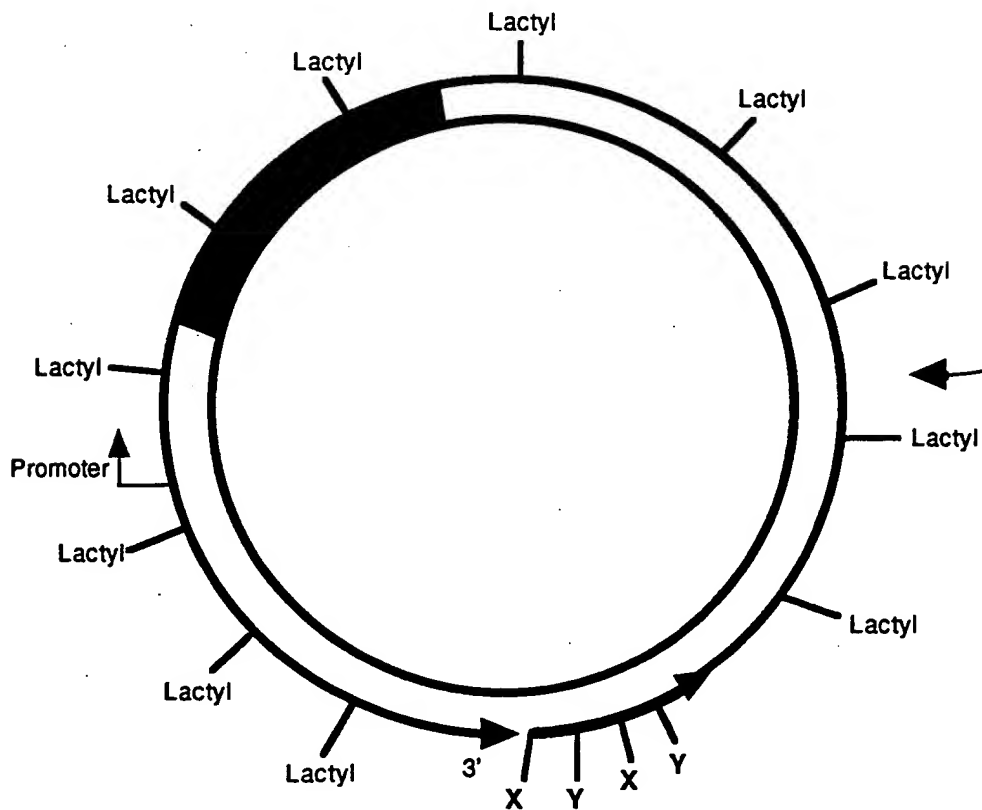
(a)

X = Nuclear Localisation Signal

Y = fusogenic peptide

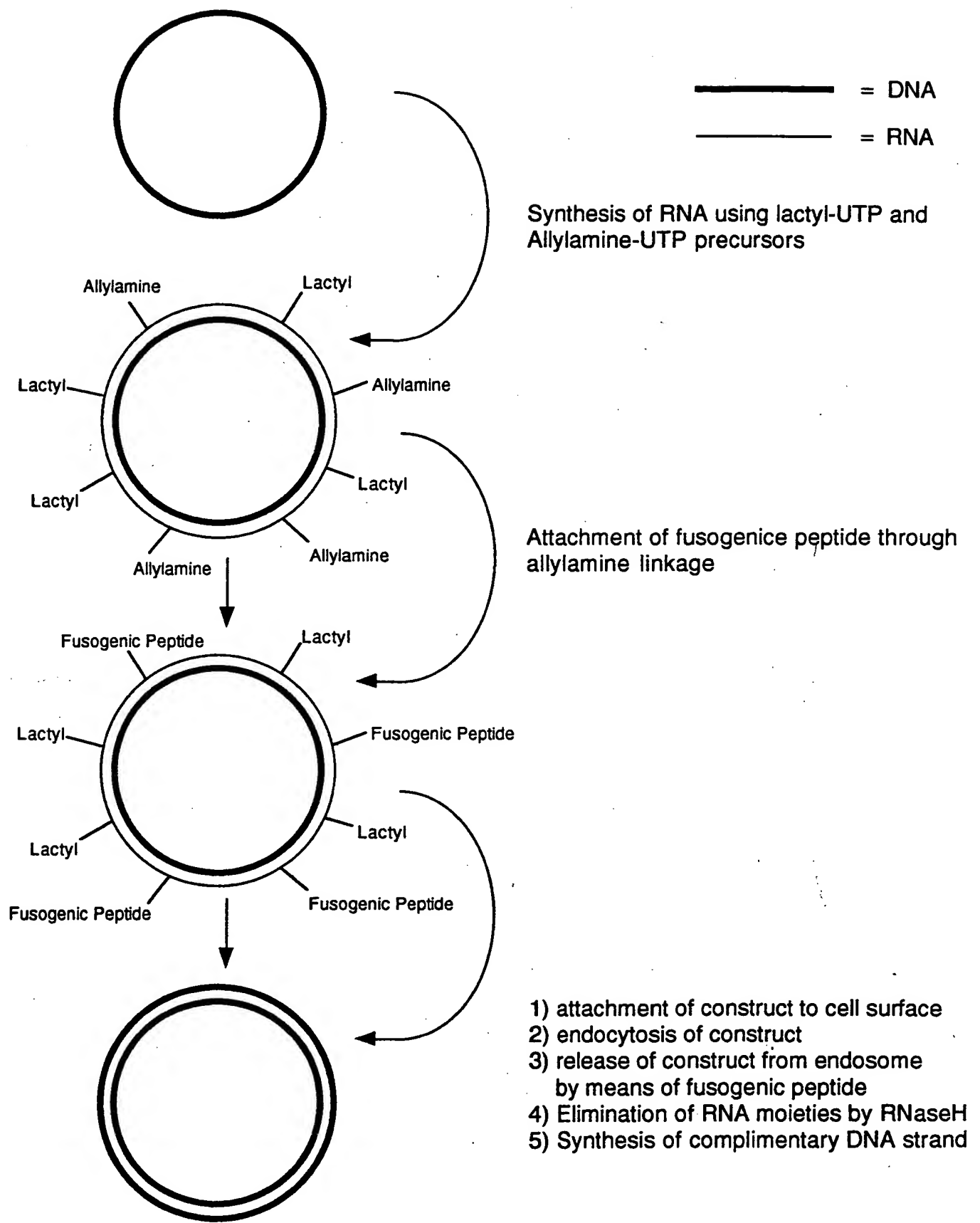


(b)

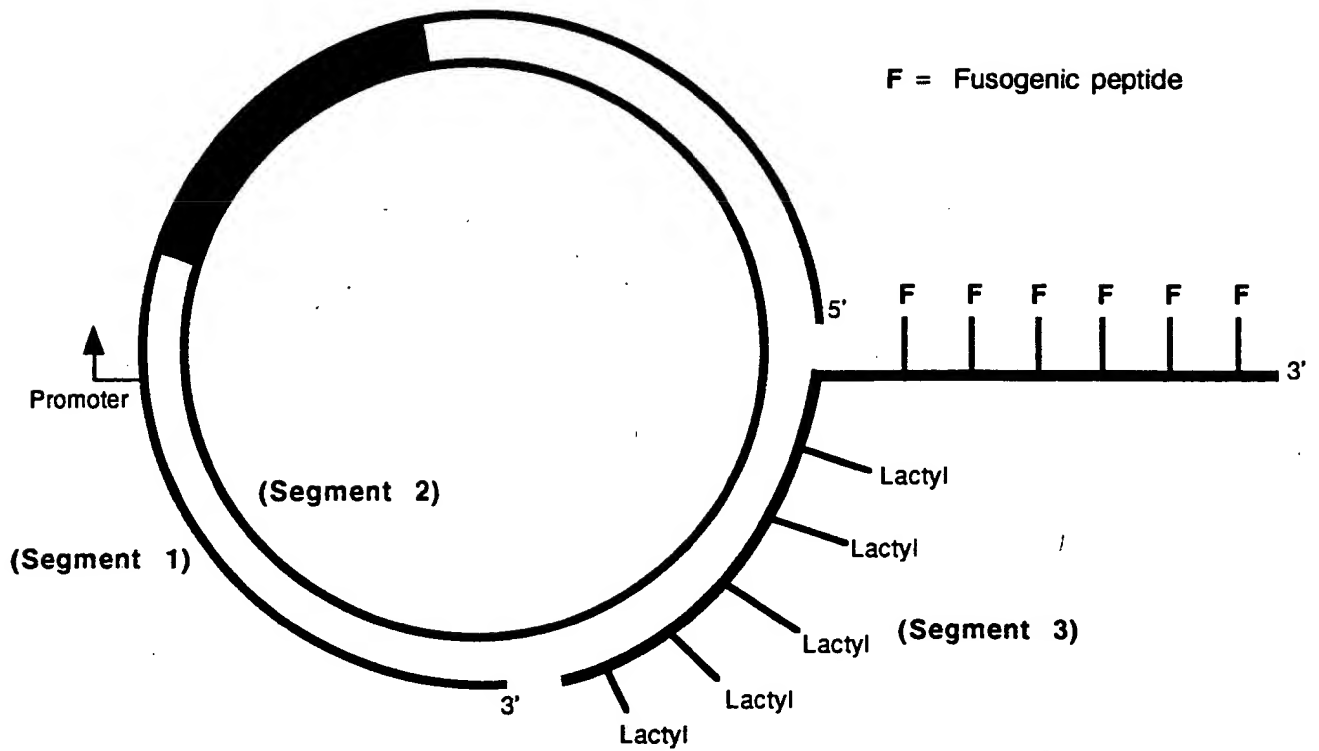
**Figure 2**

Attachment of Ligands by Incorporation of  
Modified Nucleotide Precursors

00978337 442597

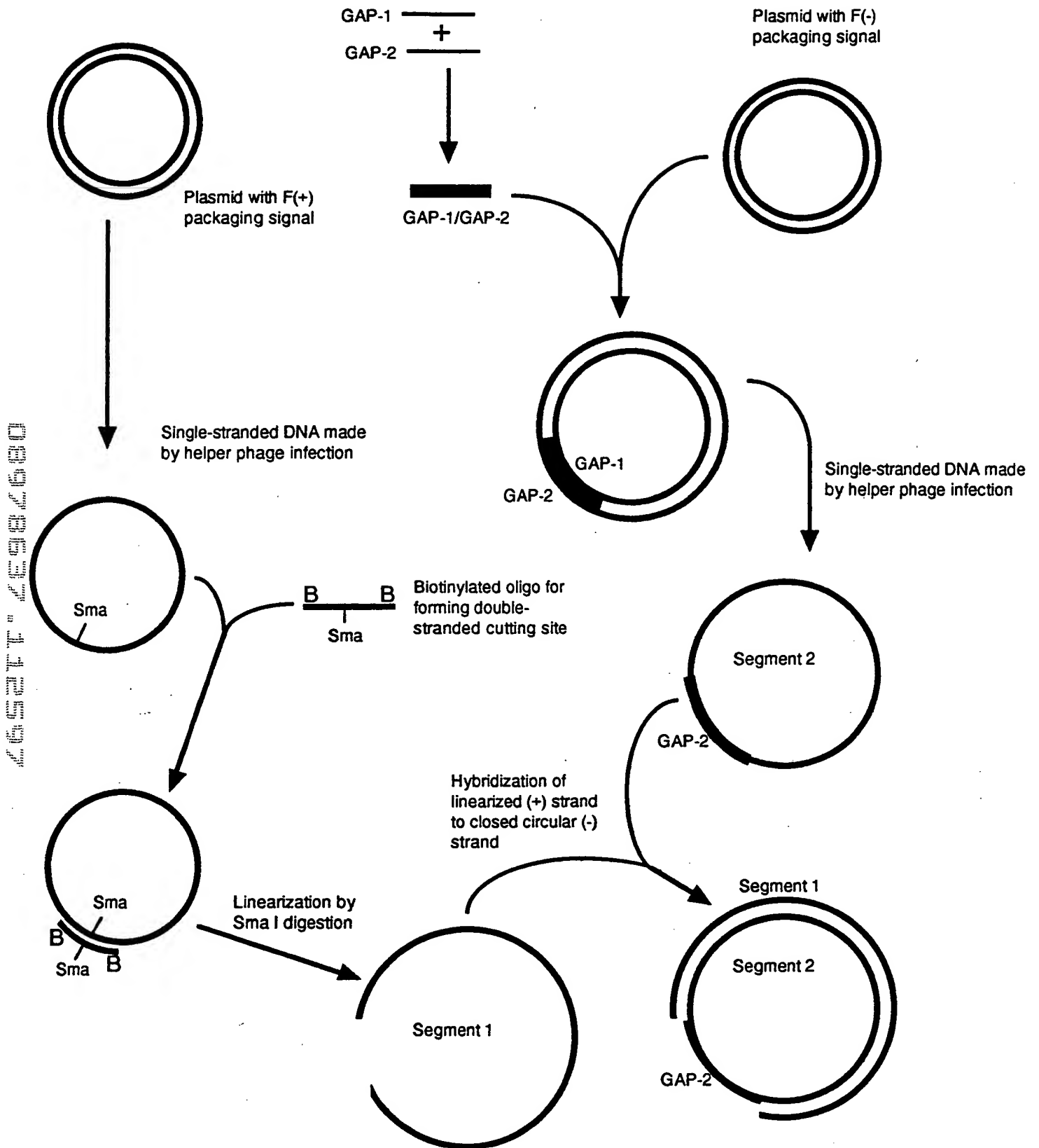


**Figure 3**  
Incorporation of Ligands through Modified Ribonucleotides

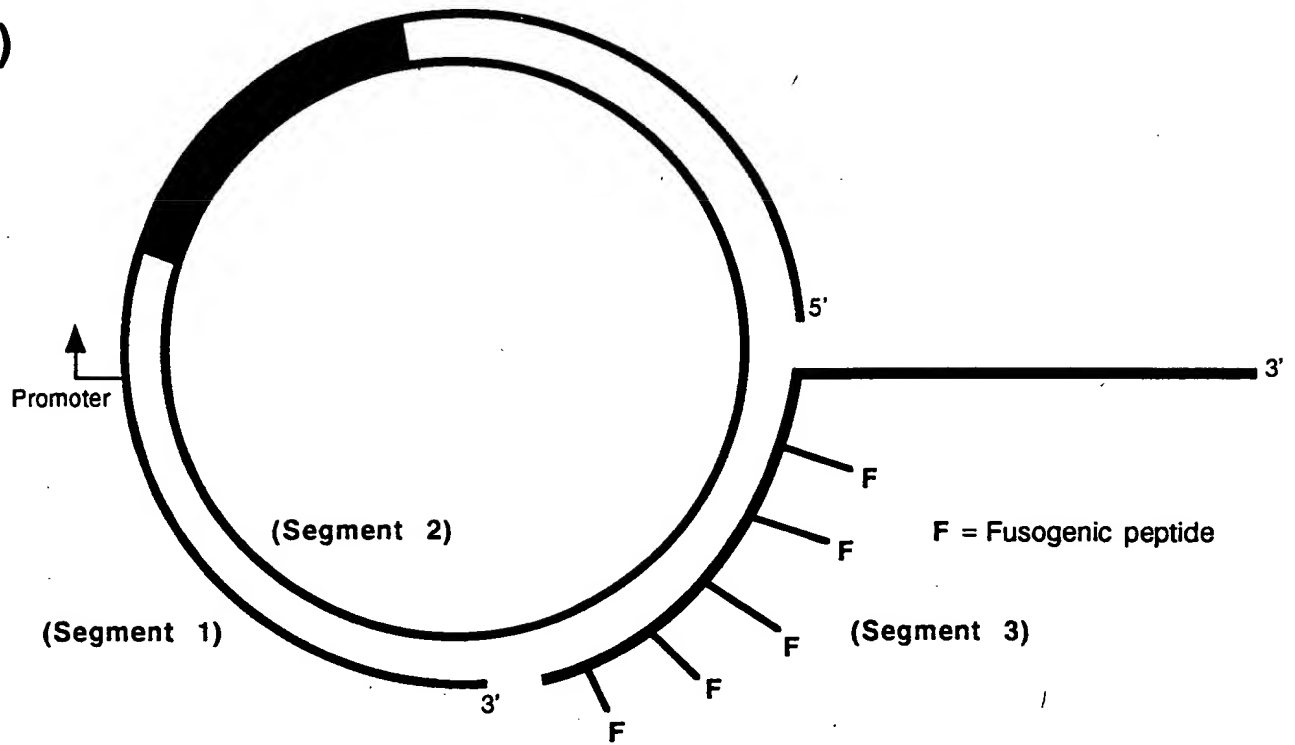


**Figure 4**

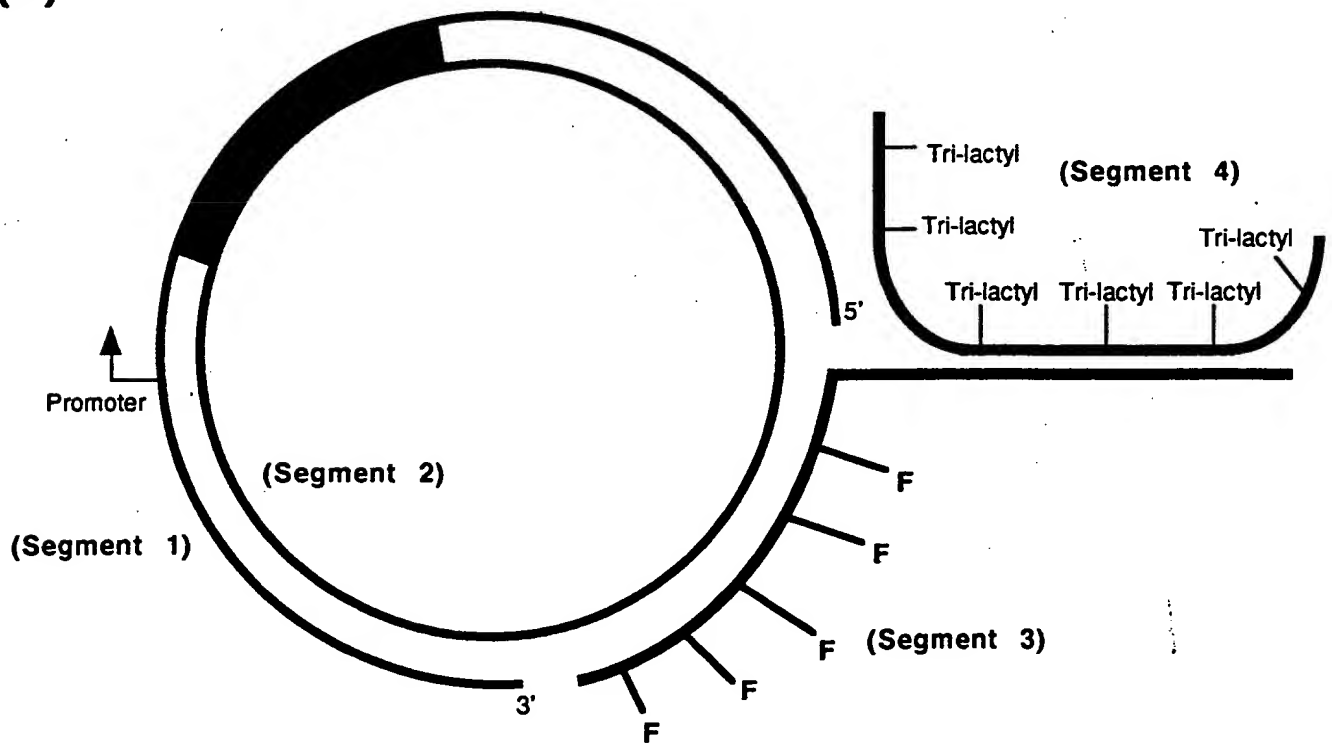
Attachment of Ligands through a 3' tail



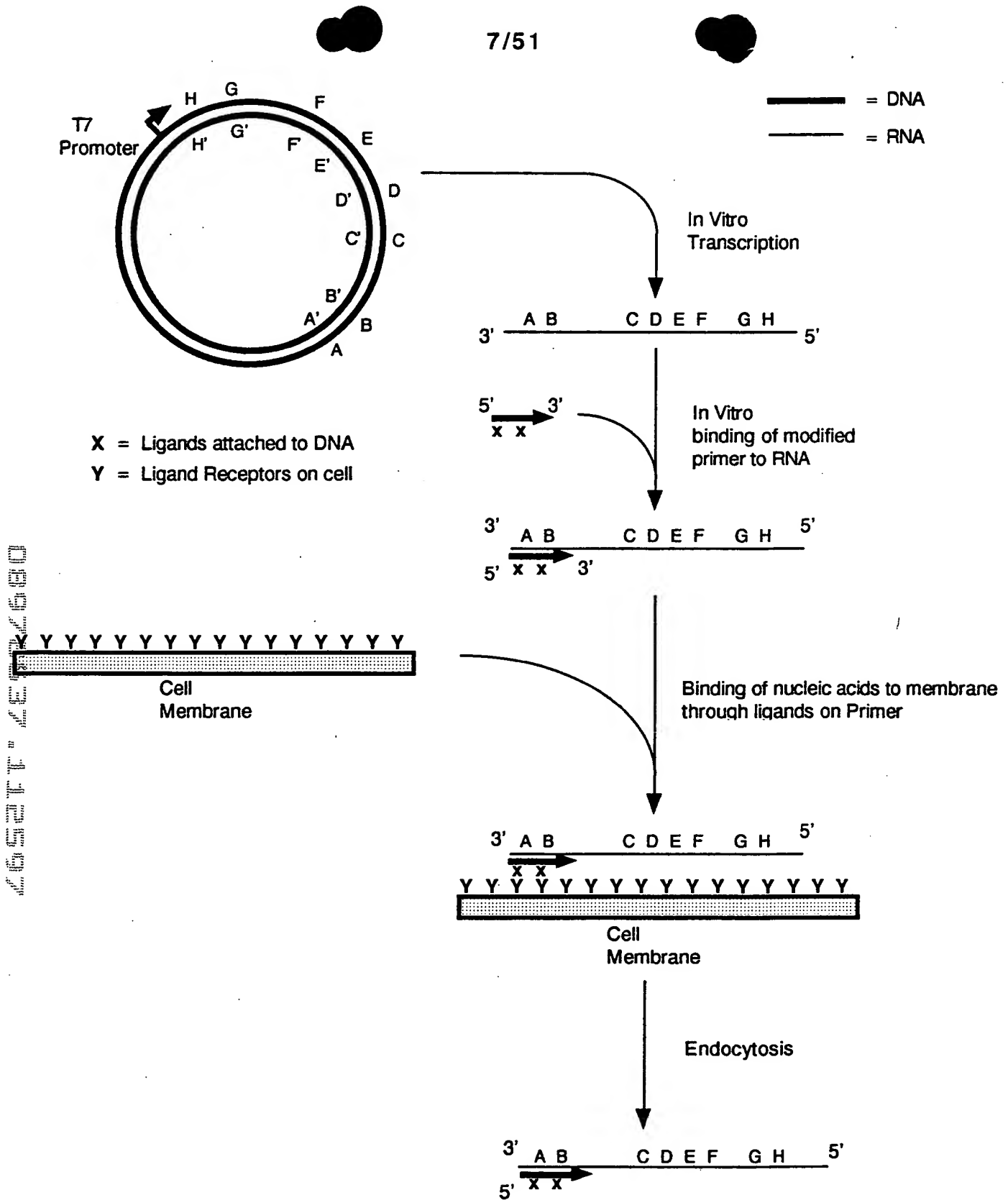
(a)



(b)

**Figure 6**

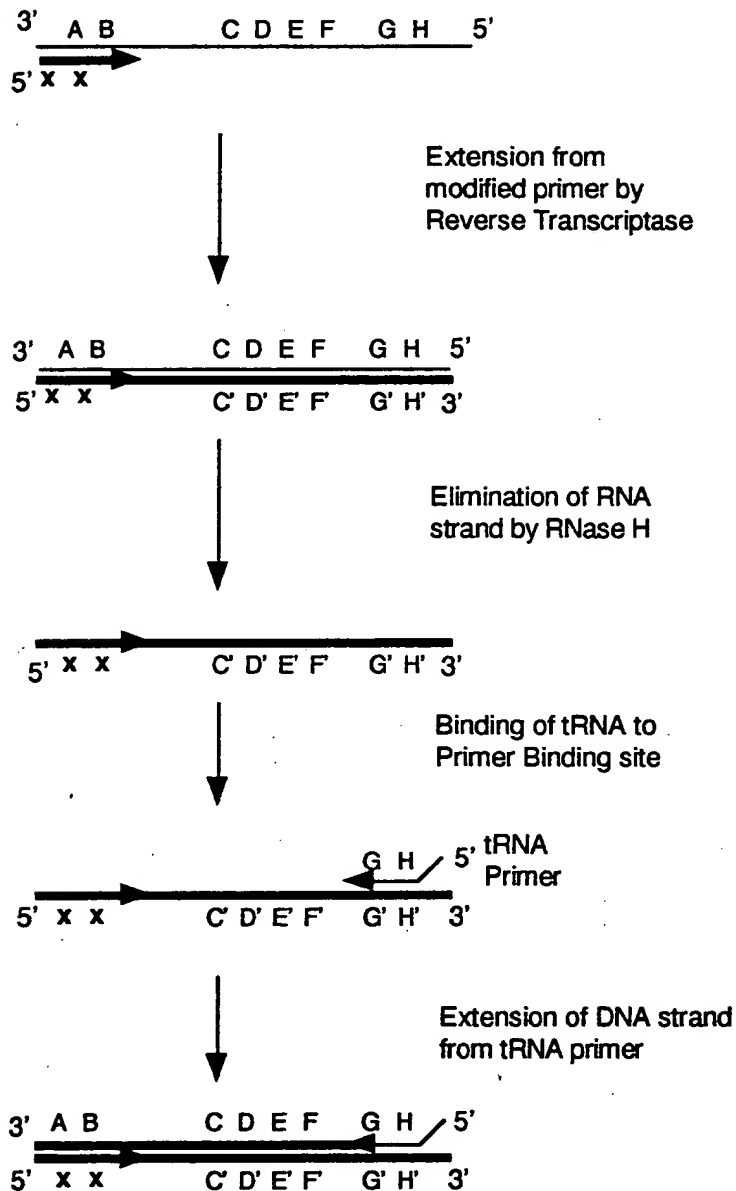
Attachment of Ligands through hybridization to a 3' tail

**Figure 7**

RNA with Ligands on Primer

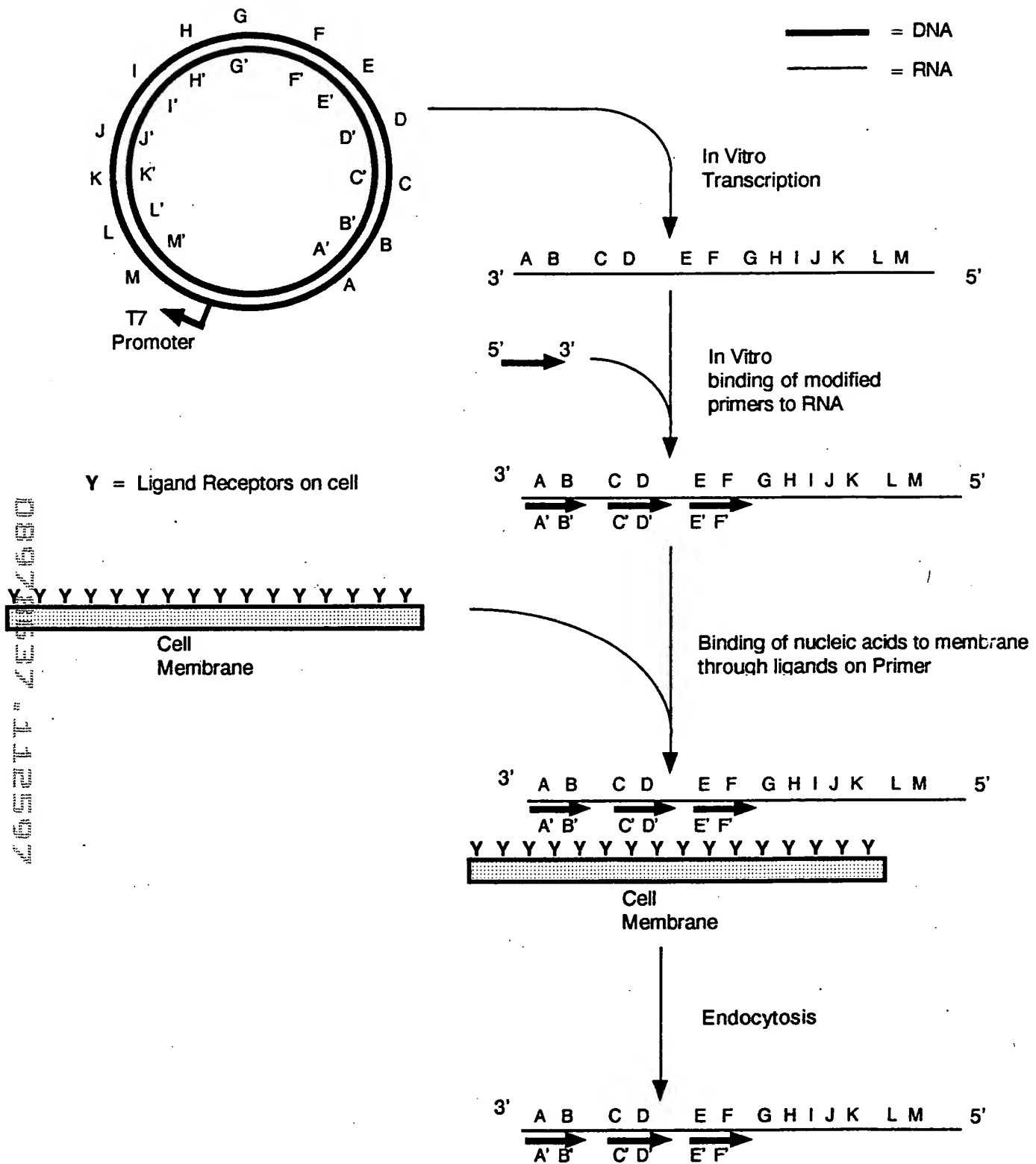
(Continued in Figure 8)

Continued from Figure 7

**Figure 8**

RNA with Ligands on Primer (Continued)





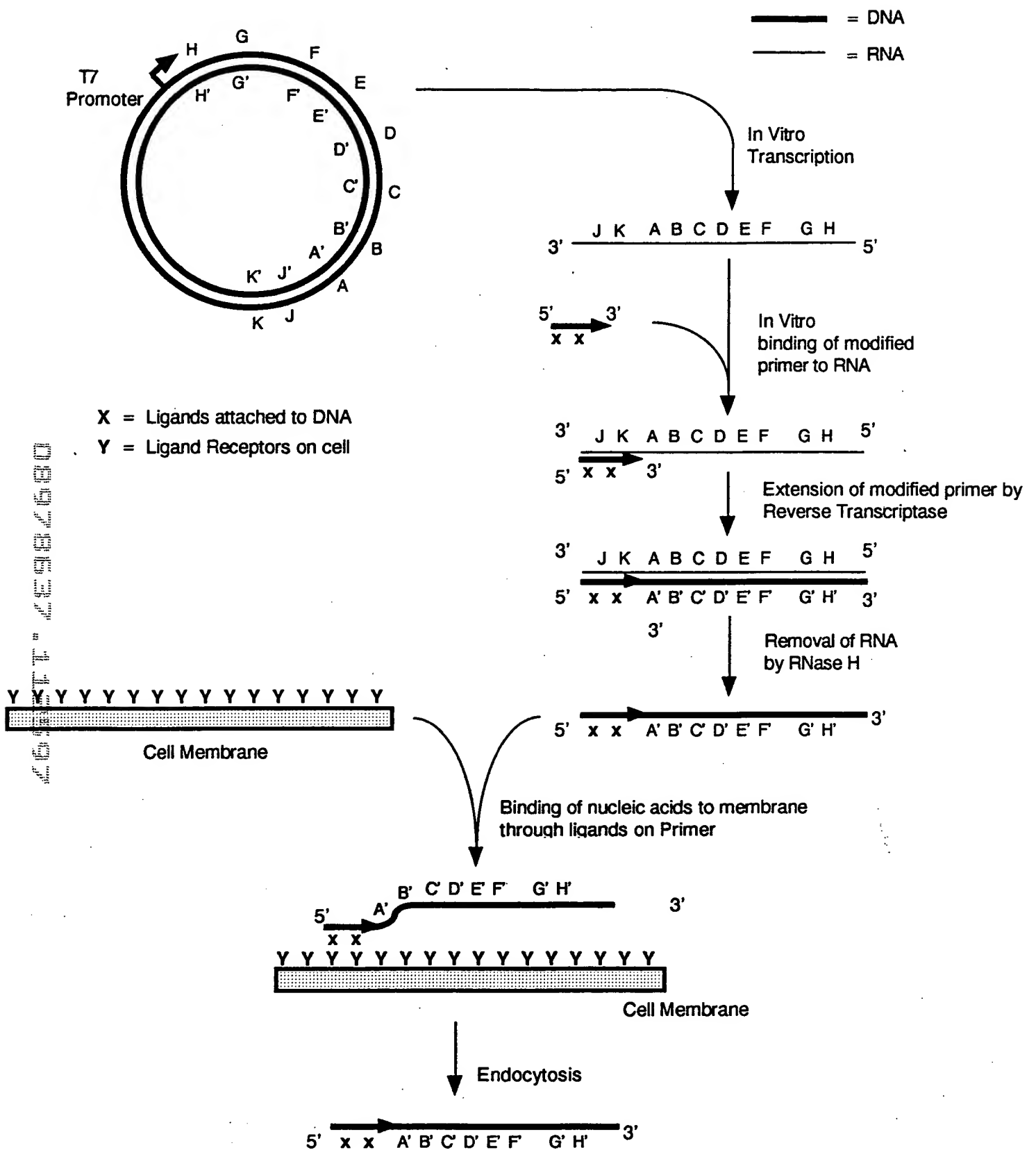
(Continued in Figure 10)

**Figure 9**  
RNA with Ligands on Multiple Primers

080906Z JUL 77



## RNA with Ligands on Multiple Primers (Continued)



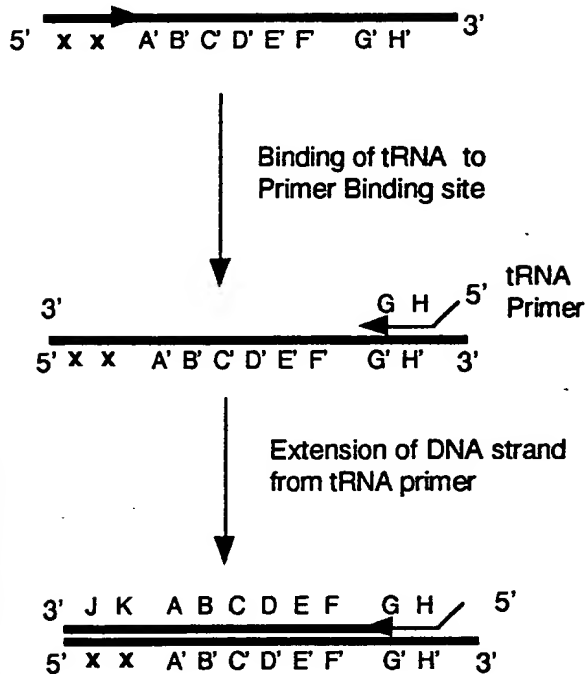
(Continued in Figure 12)

**Figure 11**  
Single-stranded DNA with attached Ligands

Continued from Figure 11

(a)

Presence of a single  
tRNA primer site



(b)

Presence of multiple  
tRNA primer sites

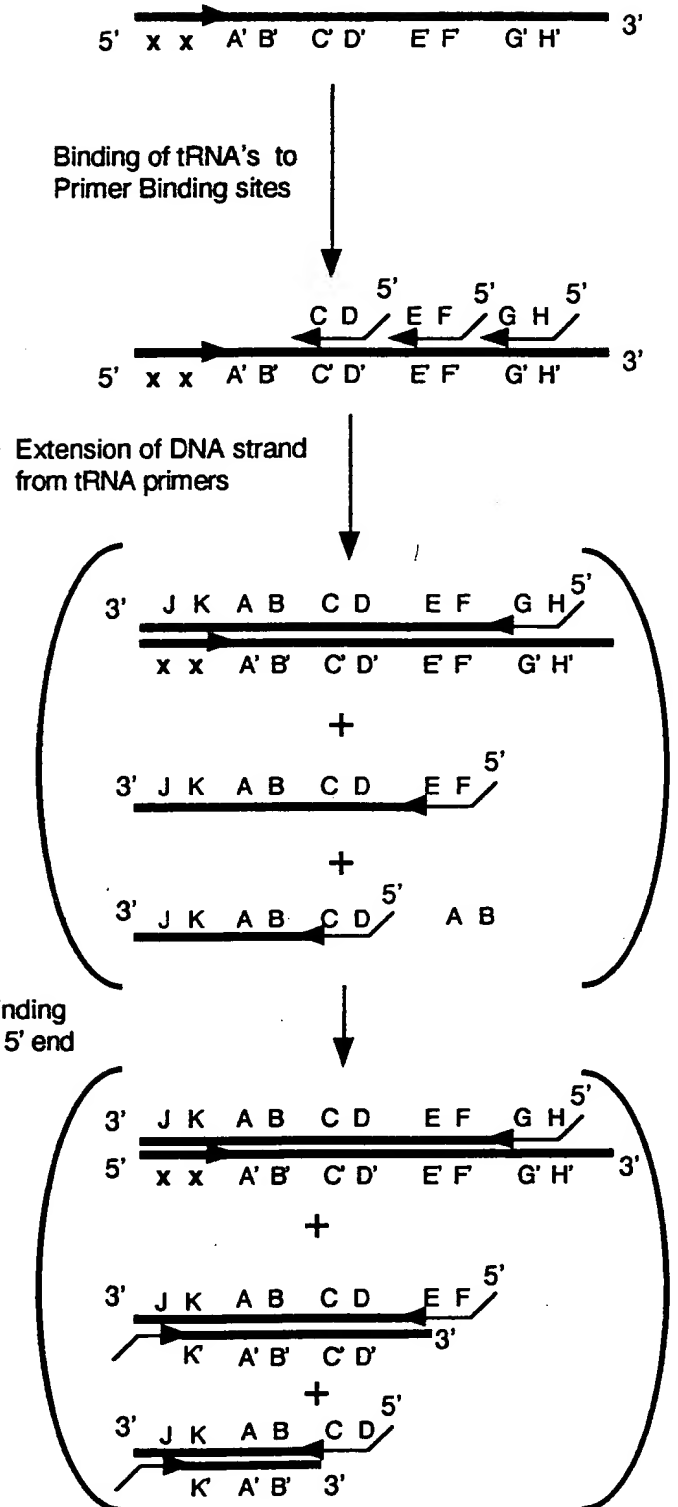
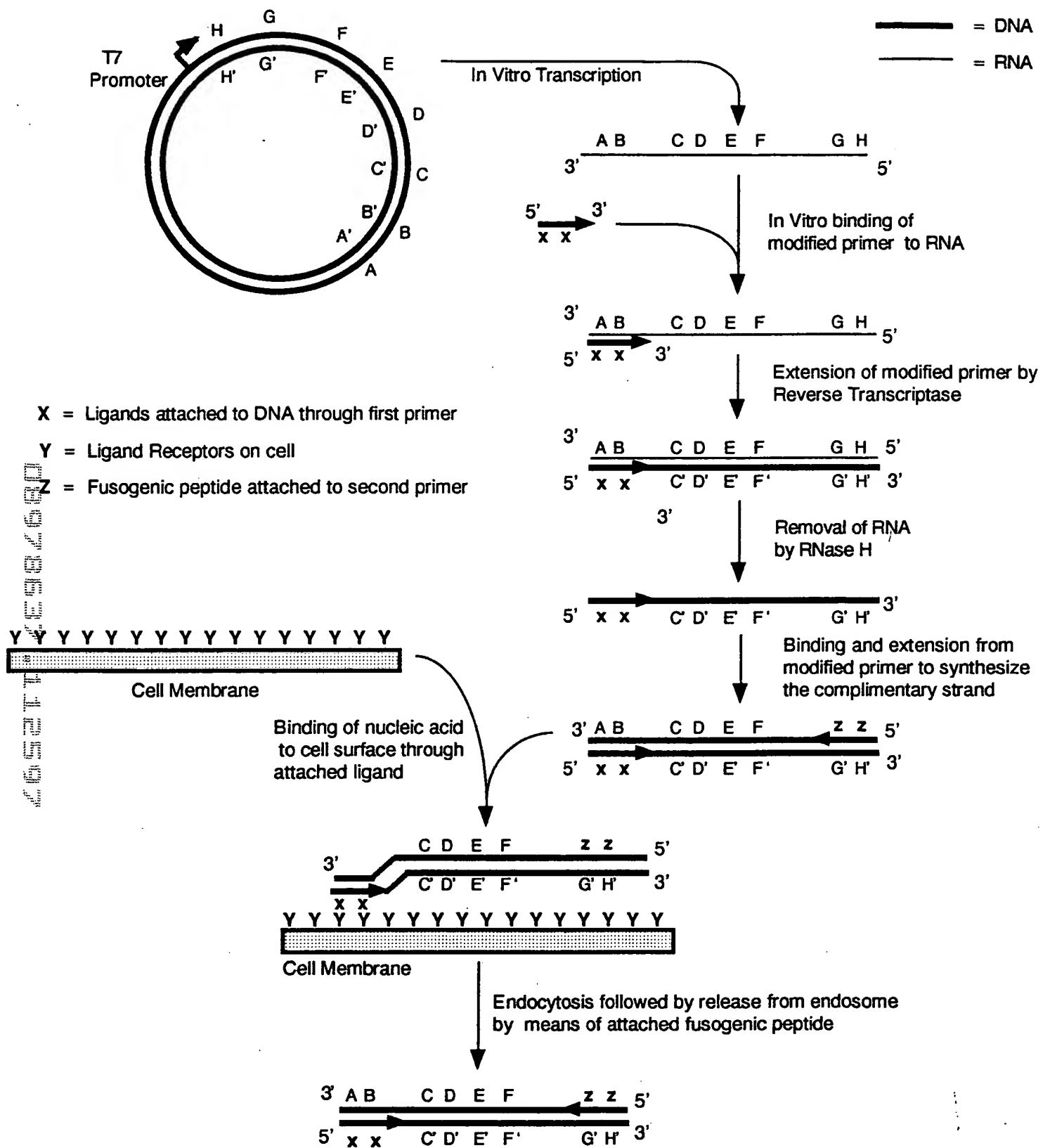


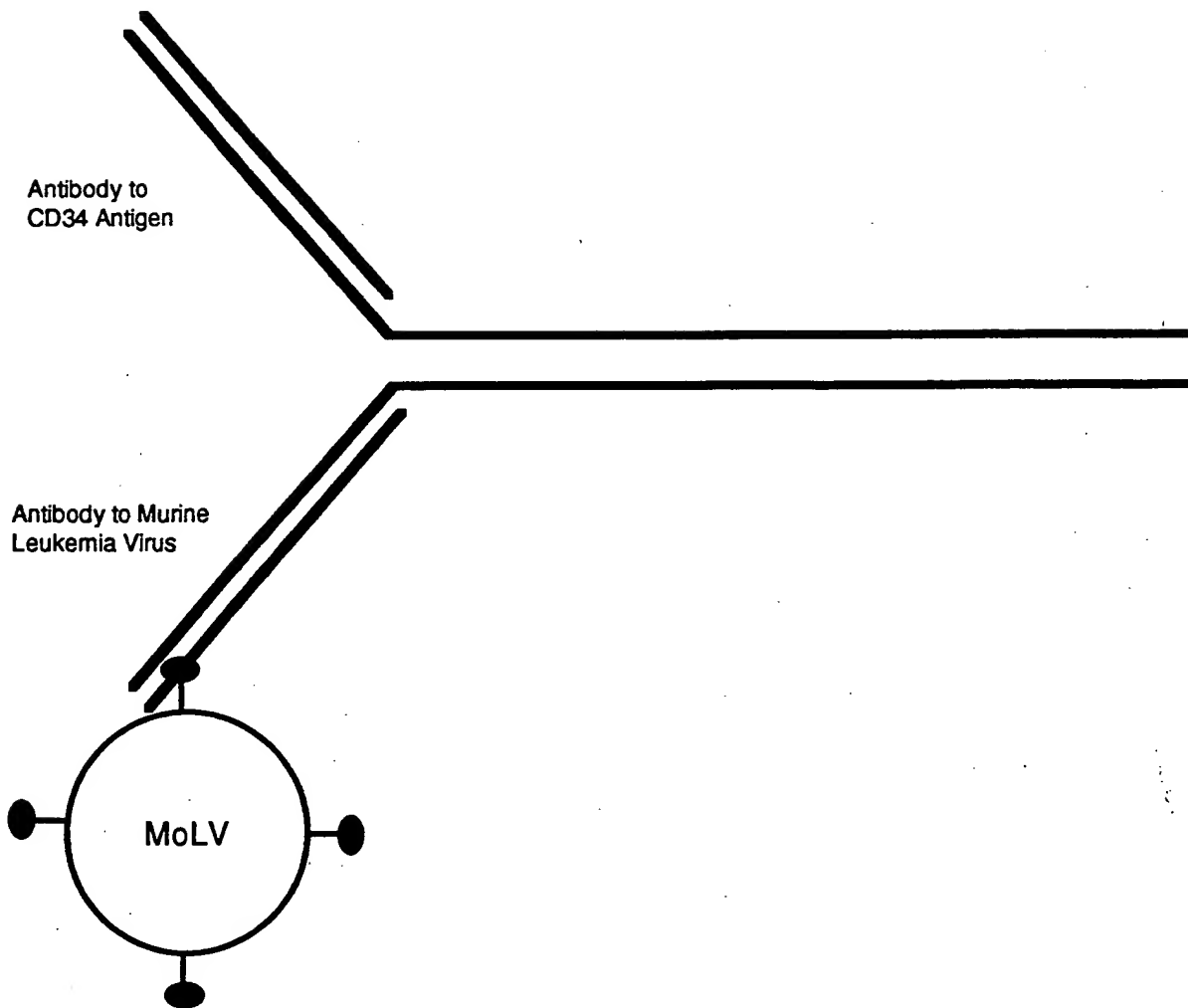
Figure 12

Single-stranded DNA with attached Ligands (continued)

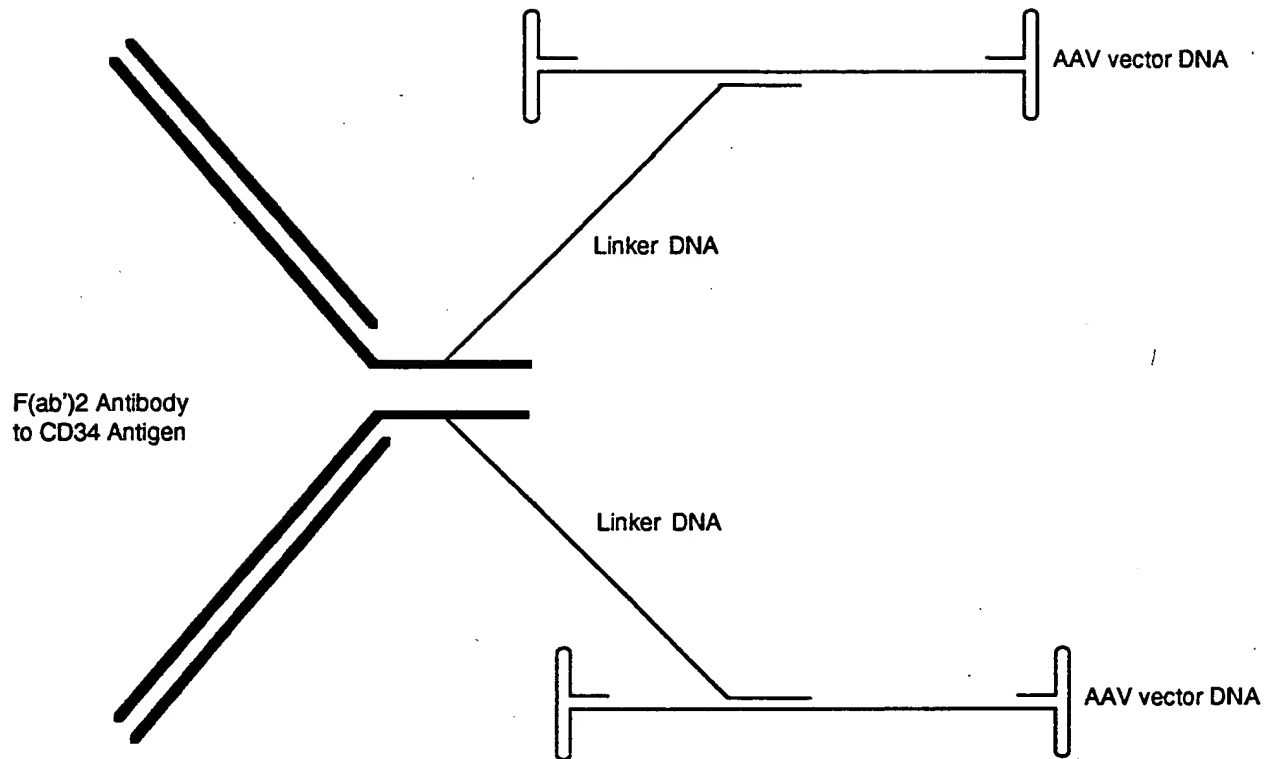
**Figure 13**

Linear Double-stranded DNA with attached Moieties on each strand

08978637 11263 465217

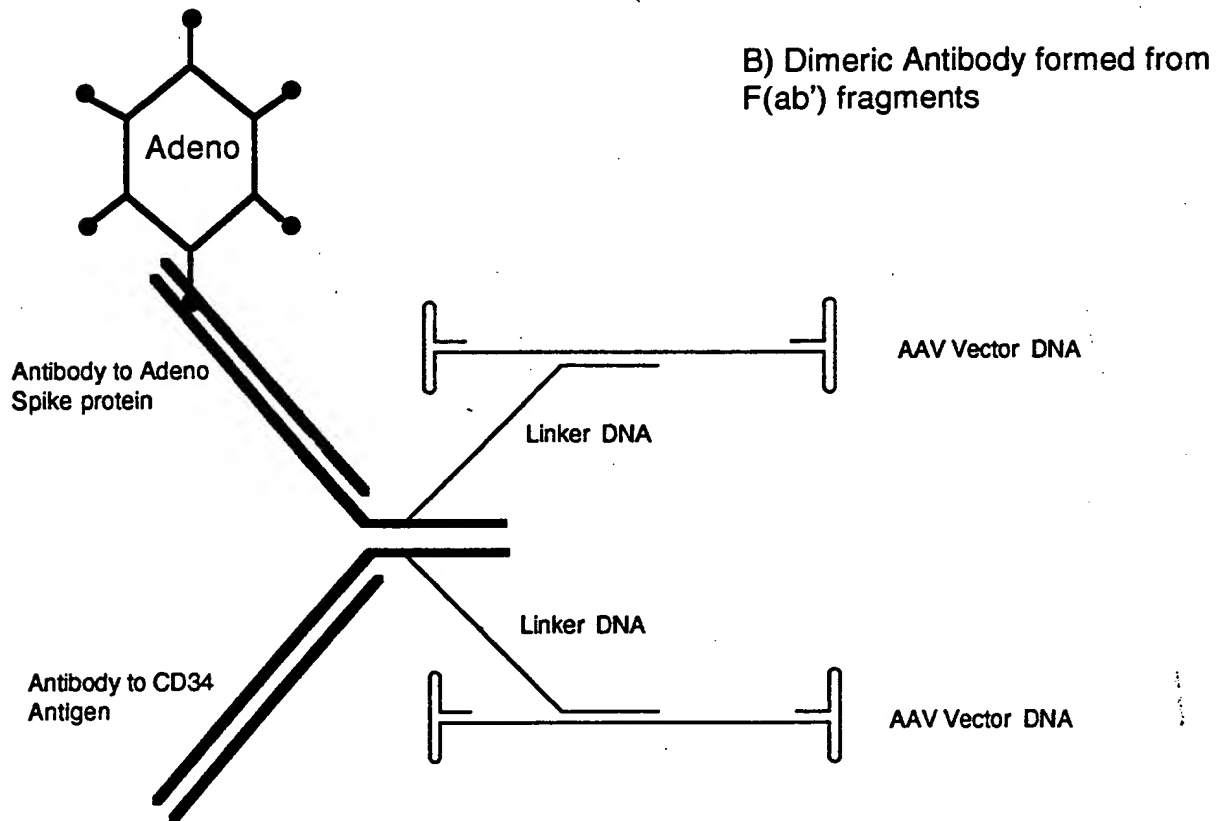
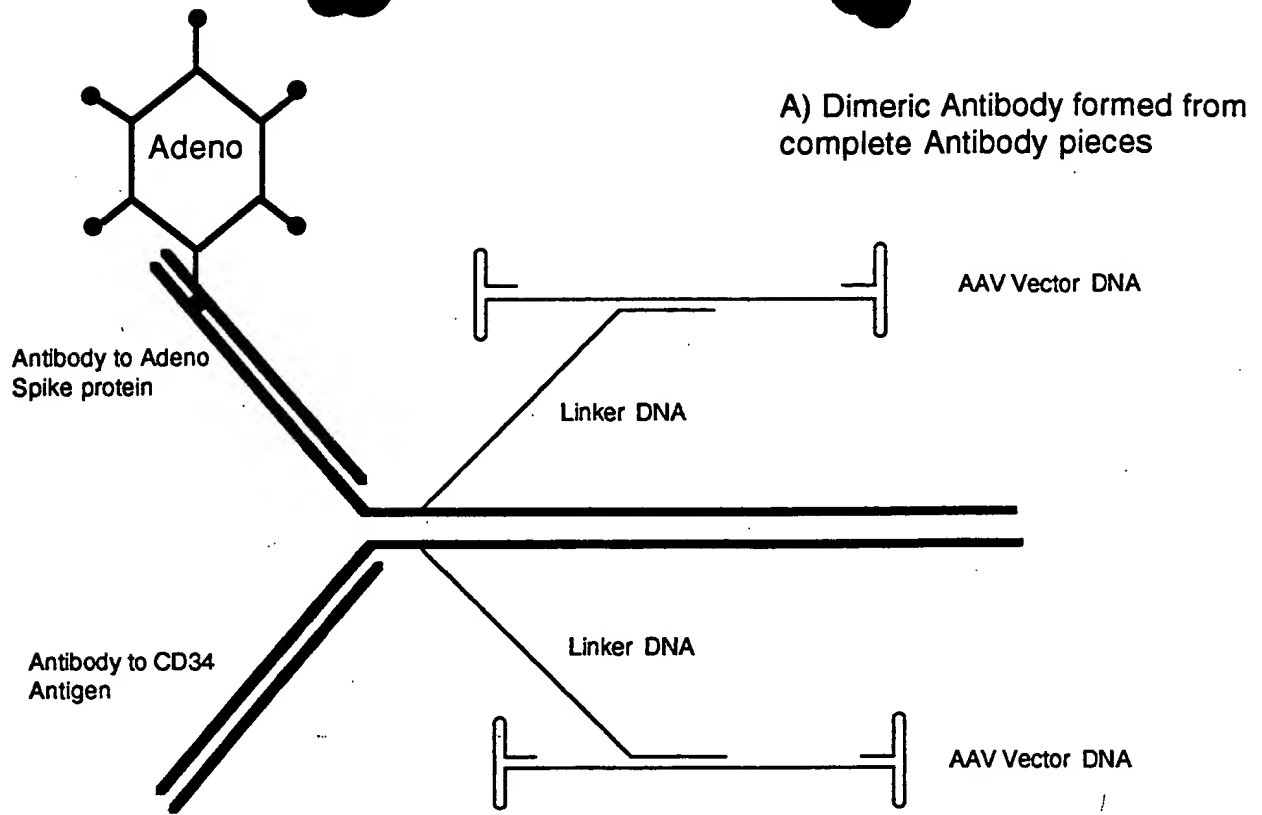
**Figure 14**

Enhanced Delivery of Retroviral Vector  
to Haematopoietic Stem Cell



**Figure 15**

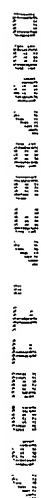
Enhanced Delivery of Vector  
DNA to Haematopoietic Stem Cell



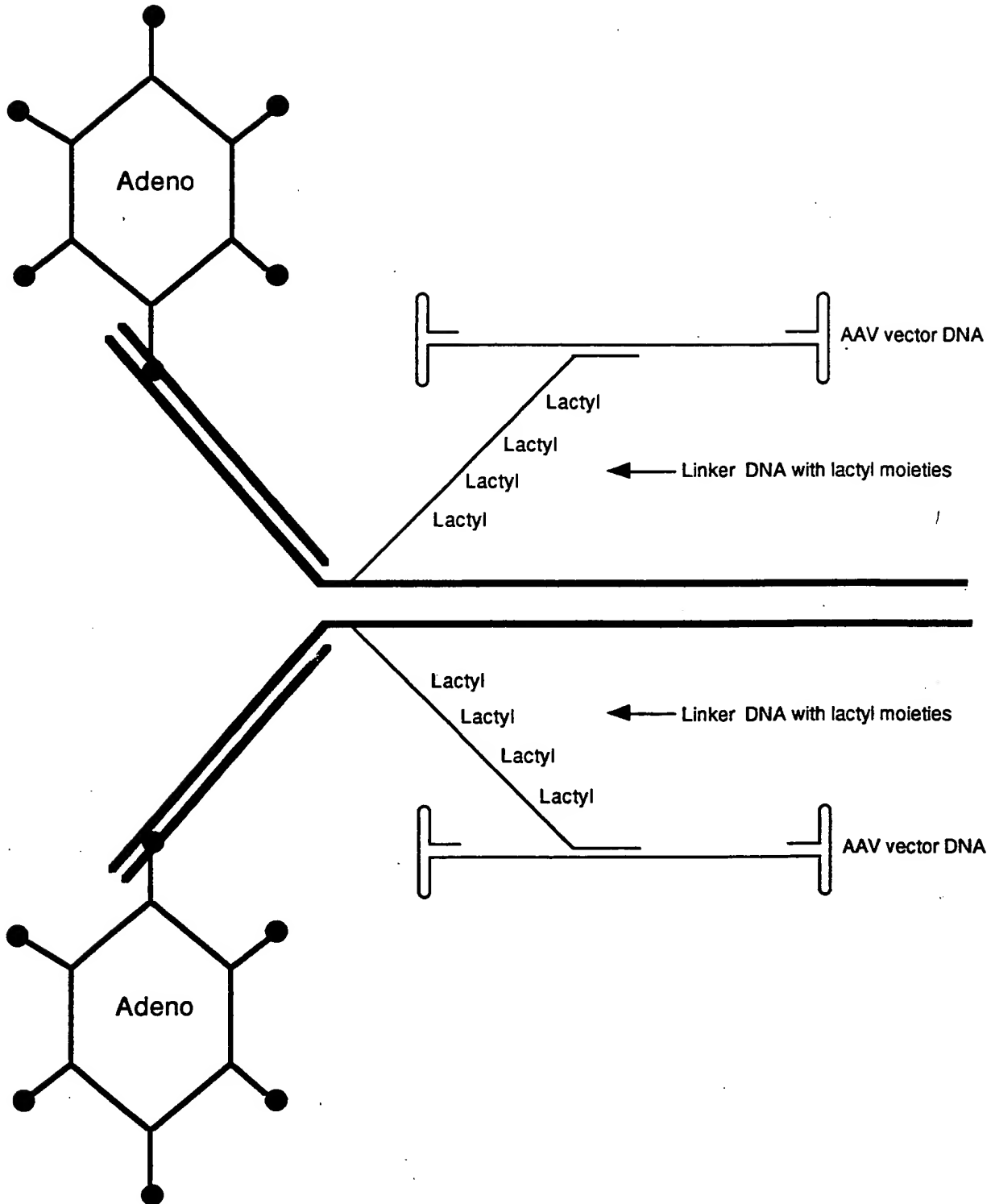
**Figure 16**

Covalent Attachment of vector DNA to Dimeric Antibody

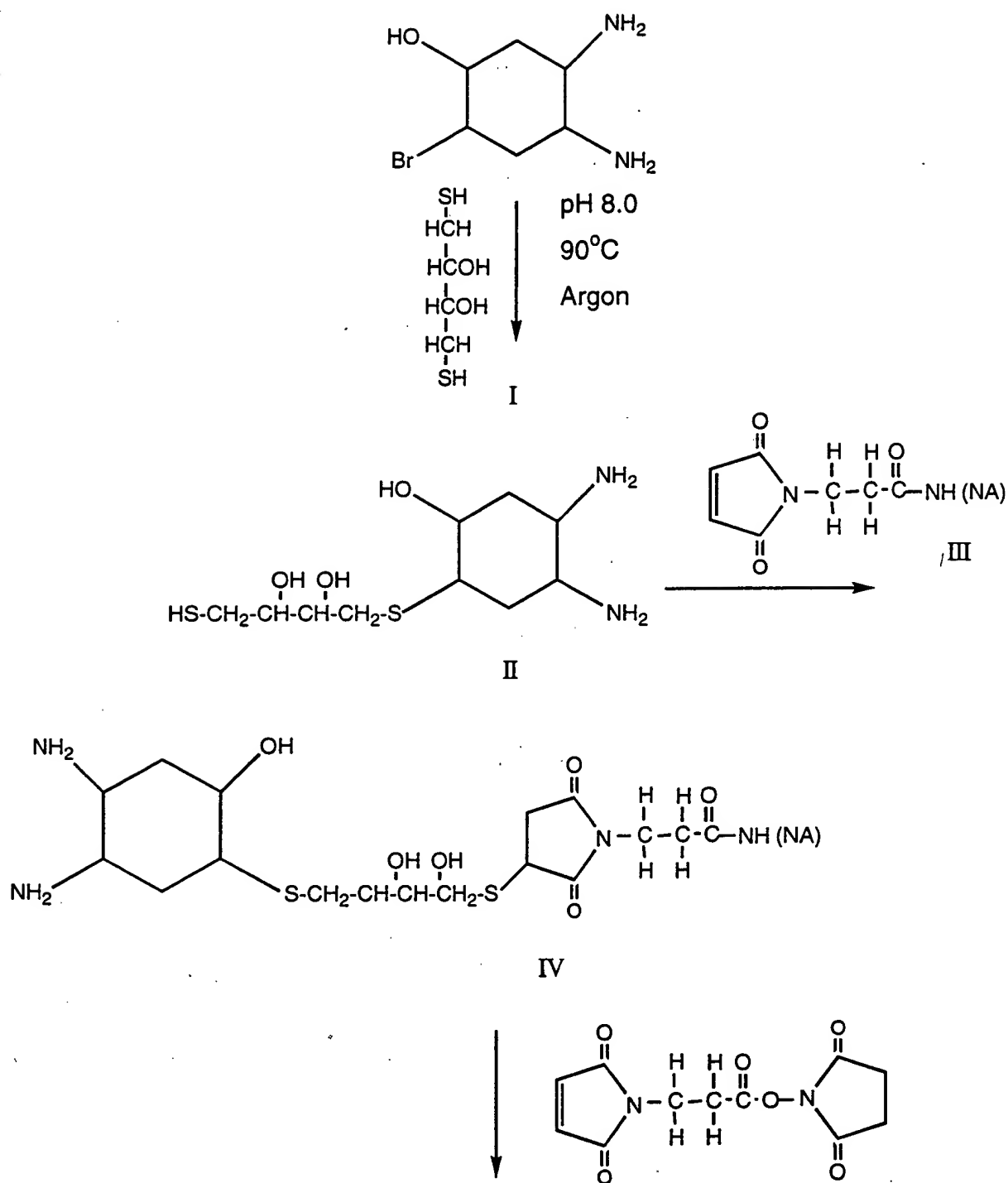




**Figure 17**  
**Covalent attachment of Modified DNA**  
**to a Monovalent Antibody**



**Figure 18**  
Modified DNA used as a Binder

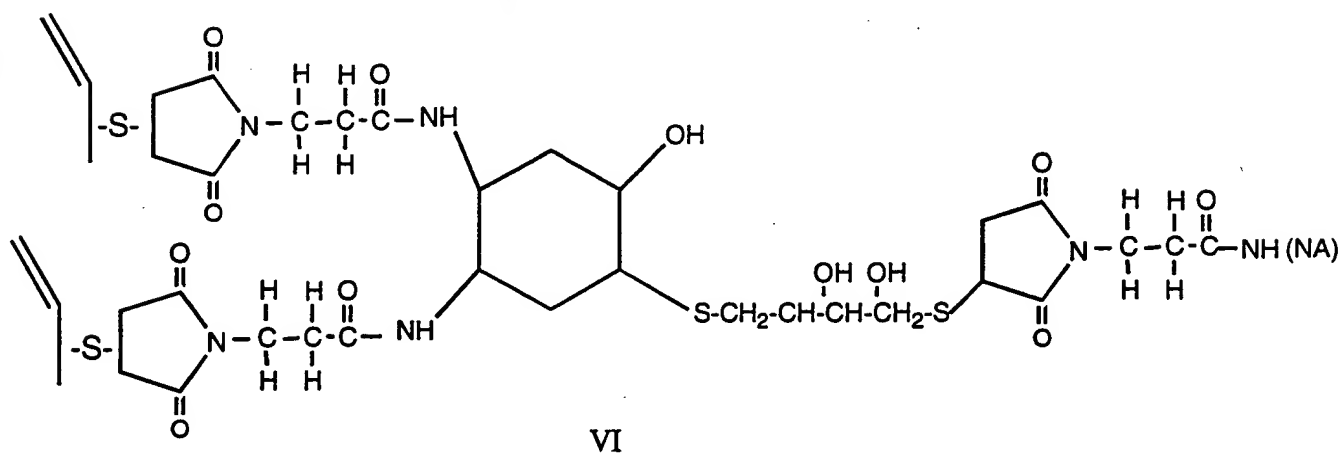
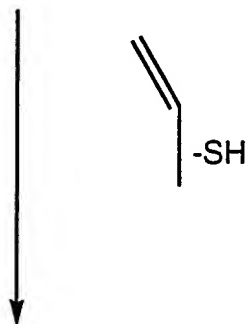
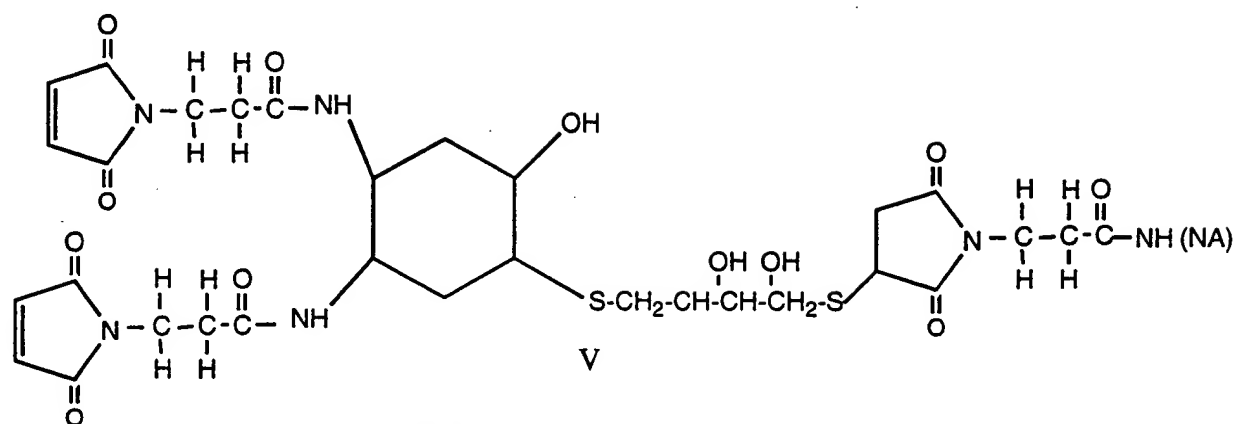


(continued in Figure 20)

**Figur 19**  
**Synthetic Steps for Creation of Antibodies**  
**With Nucl ic Acid Moieties Attach d**

(Continued from Figure 19)

20/51



**Figure 20**  
**Continuation of Synthetic Steps**

F(ab')<sub>2</sub> Antibody  
to HIV p24  
Antigen

F(ab')<sub>2</sub> Antibody  
with Nucleic Acid  
homopolymer

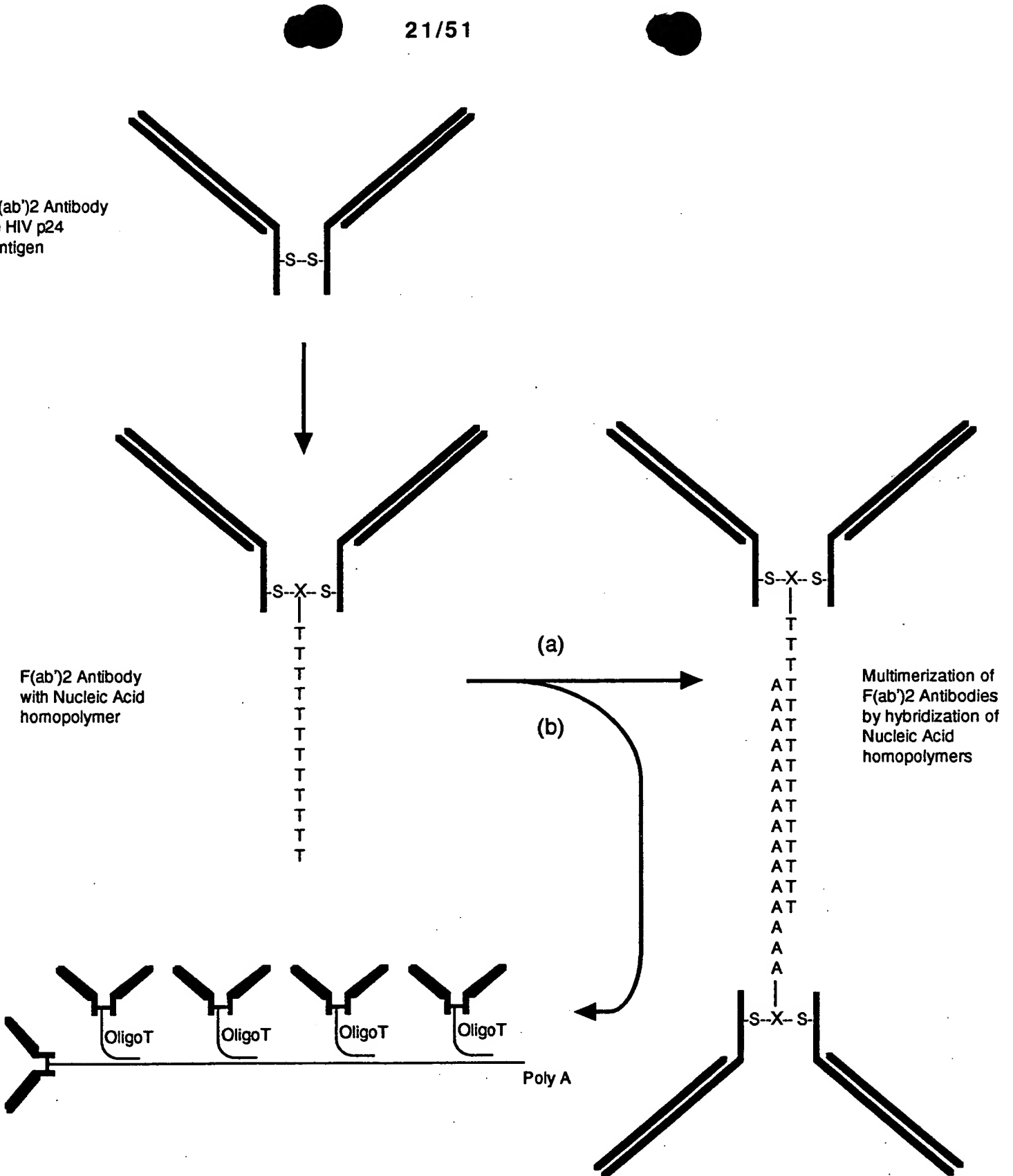
Multimerization of  
F(ab')<sub>2</sub> Antibodies  
by hybridization of  
Nucleic Acid  
homopolymers

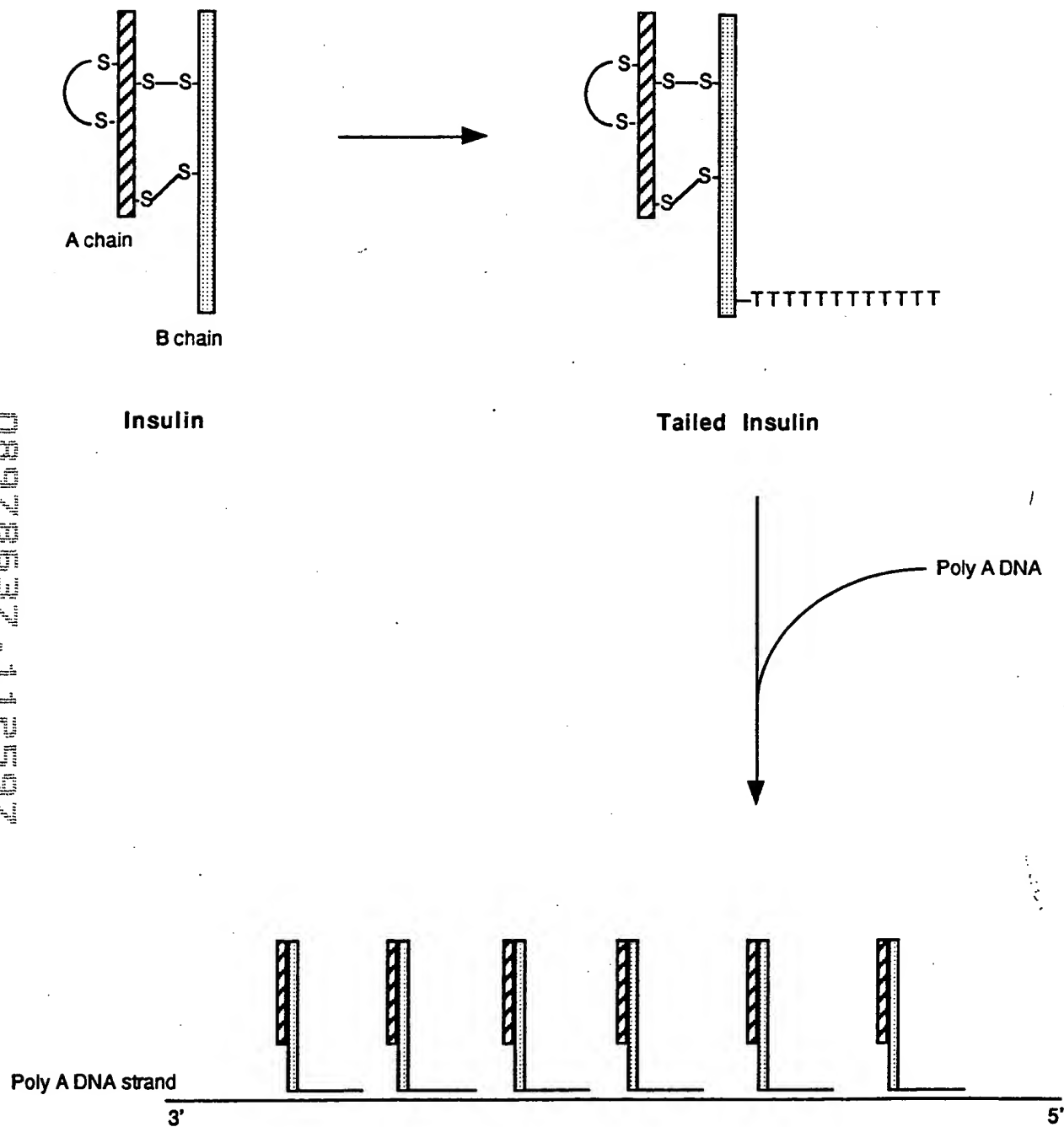
Poly A

Figure 21

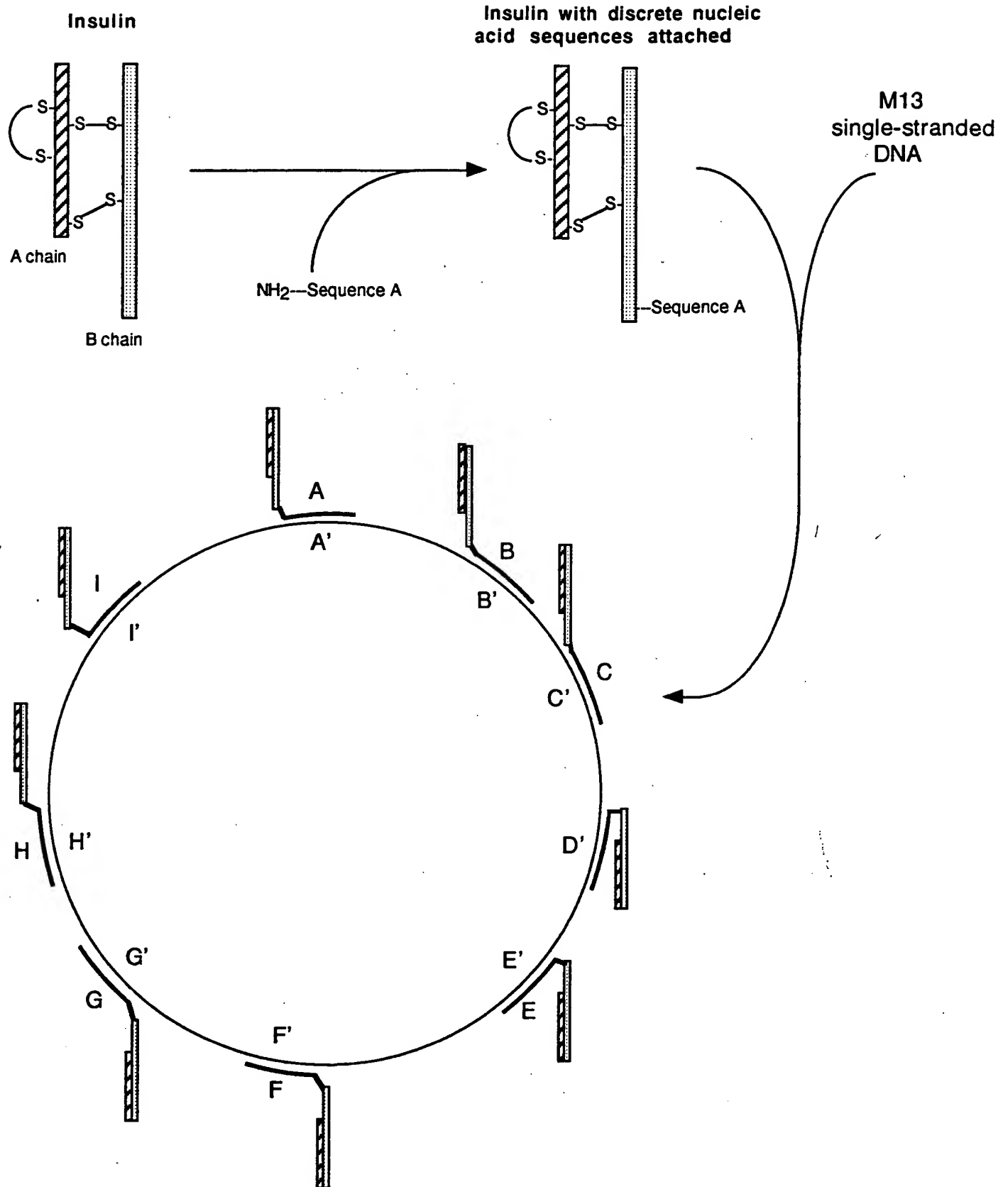
Enhanced Binding of Antibodies to Antigens by Multimerization

08078637-11297



08978637 112597  
/65211" /E98/680

**Figure 22**  
**High Affinity Multi-Insulin Soluble Complex**



**Figure 23**

Multimerization of Insulin molecules by hybridization to discrete Sequences

08978637-112597

Intron insertion site

(A)    **-----TGCTCTCTAAGGGTCTACTC-----**  
          **-----ACGAGAGATTCCCAGATGAG-----**

T7 RNA Polymerase Sequence

Splice Donor Site

Splice Acceptor site

(B)    **-----CTCTAAGGTAAATAT-----TGTATTTTAGATTCAA-----**  
          **-----GAGATTCCATTATA-----ACATAAAATCTAAGTT-----**

SV40 Intron Sequence

(C)    **-----TGCTCTCTAAGGTAAATAT-----TGTATTTTAGGGTCTACTC-----**  
          **-----ACGAGAGATTCCATTATA-----ACATAAAATCCCAGATGAG-----**

Insertion of SV40 Intron into polymerase coding sequence

(D)    **-----UGCUCUCUAAGGUAUAUAU-----UGUAUUUUAGGGUCUACUC---**

mRNA transcript containing intron

(E)    **-----UGCUCUCUAAGGGUCUACUC---**

mRNA transcript after splicing has normal T7 Sequence

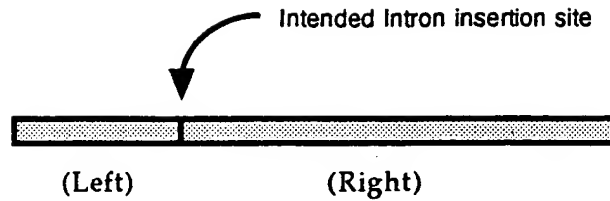
## Figure 24

### Fusion of Intron into T7 RNA Polymerase Coding Sequence

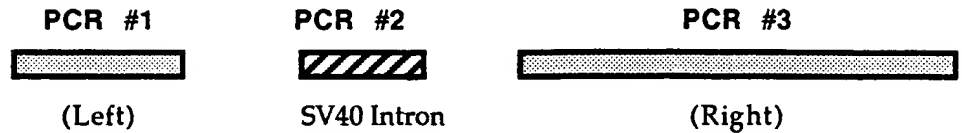
08978637-112597



Normal T7 RNA polymerase coding sequence

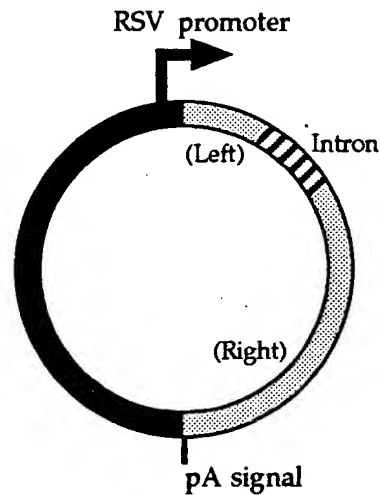


Synthesis of fragments by PCR Amplification of T7 or SV40 templates



(A)

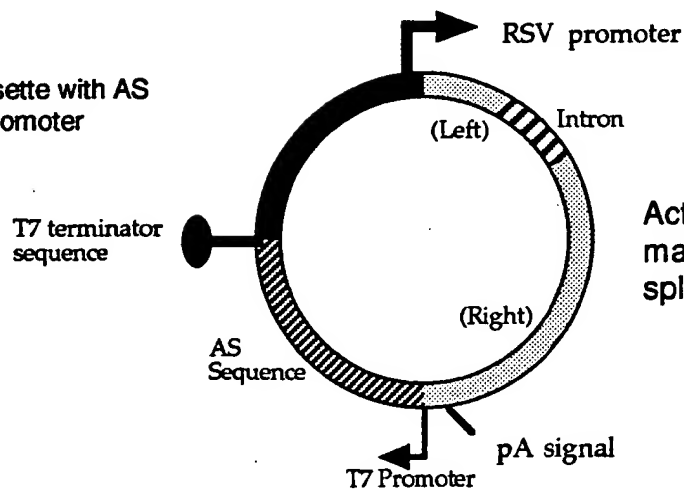
Fusion of PCR fragments together in eucaryotic expression vector



(B)

Introduction of cassette with AS directed from T7 promoter

(C)

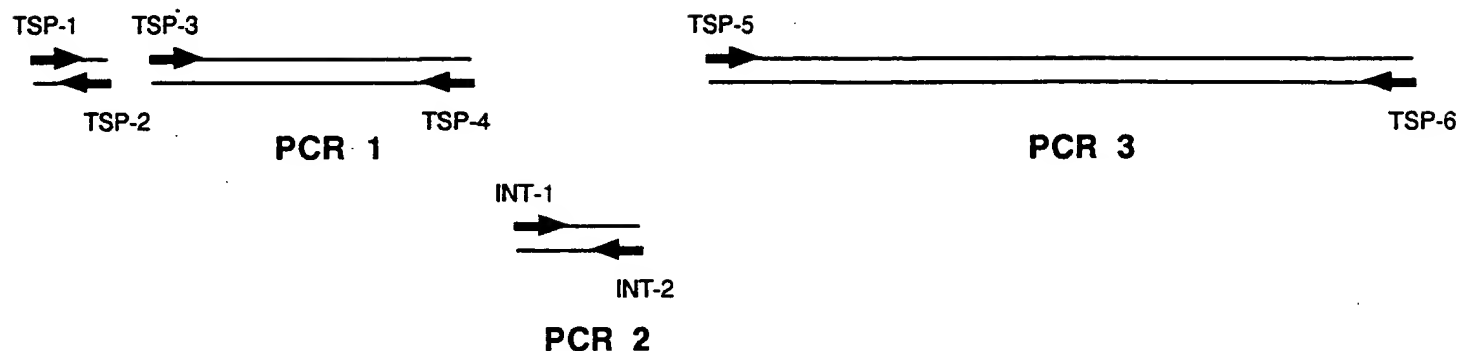


Active T7 RNA polymerase is only made in eucaryotic cells after splicing out of SV40 Intron

**Figure 25**

**Construction of T7 Expression Vector**

## A) Synthesis of pieces



## B) Oligomers used for synthesis

TSP-1	GGA ATT CGT CTC GAG CTC TGA TCA CCA CCA TGG ACA CGA TTA ACA TCG C
TSP-2	GAC TAG TTG GTC TCG TCT CTT TTT TGG AGG AGT GTC GTT CTT AGC GAT GTT AAT C
TSP-3	GGA ATT CGT CTC GGA GAA AGG TAA AAT TCT CTG ACA TCG AAC TGG C
TSP-4	GAC TAG TGG TCT CCC CTT AGA GAG CAT GTC AGC
TSP-5	GGA ATT CGG TCT CGG GTC TAC TCG GTG GCG AGG
TSP-6	GAC TAG TCG TTA CGC GAA CGC AAA GTC
INT-1	GGA ATT CGT CTC TAA GGT AAA TAT AAA ATT TTT AAG
INT-2	GAC TAG TCG TCT CTG ACC CTA AAA TAC ACA AAC AAT TAG A

**Figure 26**

Synthesis of Pieces for Construction of  
T7 RNA Polymerase with Intron

# Formation of Nuclear Localisation Signal by Fusion of TSP1/TSP2 Product to Clone with PCR #1 product

## Annealing of TSP1 with TSP2

TSP1

5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GC 3'

3' C TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT GCT CTG GTT GAT CAG 5' TSP2

## Extension of TSP1/TSP2 by polymerase

5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AAG AGA CGA GAC CAA CTA CTC 3'  
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT GAT CAG 5' Bsa I

## Digestion of TSP1/TSP2 product with Bsa I

5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AA  
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT

## Digestion of PCR #1 clone (pL-1) with BsmB I

Bsm B1  
5' GGA ATT GGT CTC G GAGA AAG GTA AAA TTC TCT GAC ATC GAA CTG GC-----  
CCT TAA GCA GAG CTTCT TTC CAT TTT AAG AGA CTG TAG CTT GAC CG-----

## Ligation of Bsa I digested TS1/TS2 product to BsmB I digested PCR#1 clone

5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AAG AGA AAG GTA AAA TTC  
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT TTC CAT TTT AAG  
TCT GAC ATC GAA CTG GC-----  
AGA CTG TAG CTT GAC CG-----

Figure 27

# Comparison of the 5' ends of the Nucleotide Sequences of Wild Type and Modified T7 RNA Polymerase

## Wild Type T7 nucleic and amino acid sequence

ATG	GAC	ACG	ATT	AAC	ATC	GCT	AGG	AAC	GAC	TTC	TCT	GAC	ATC	GAA	CTG	GC	-----
TAC	CTG	TGC	TAA	TTG	TAG	CGA	TTC	TTG	CTG	AAG	AGA	CTG	TAG	CTT	GAC	CG	-----
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		

## Modified T7 nucleic and amino acid sequence with Nuclear Localisation Signal (NLS) insertion

ATG	GAC	ACG	ATT	AAC	ATC	GCT	AGG	AAC	GAC	ACT	CCT	CCA	AAA	AAG	GTA	AAA	TTC	TCT	GAC	ATC	GAA	CTG	GC	-----		
TAC	CTG	TGC	TAA	TTG	TAG	CGA	TTC	TTG	CTG	TGA	GGA	GGT	TTT	TTT	TTC	TCT	TTC	CTT	AAG	AGA	CTG	TAG	CTT	GAC	CG	-----
1	2	3	4	5	6	7	8	9	10								11	12	13	14	15	16				

Figure 28

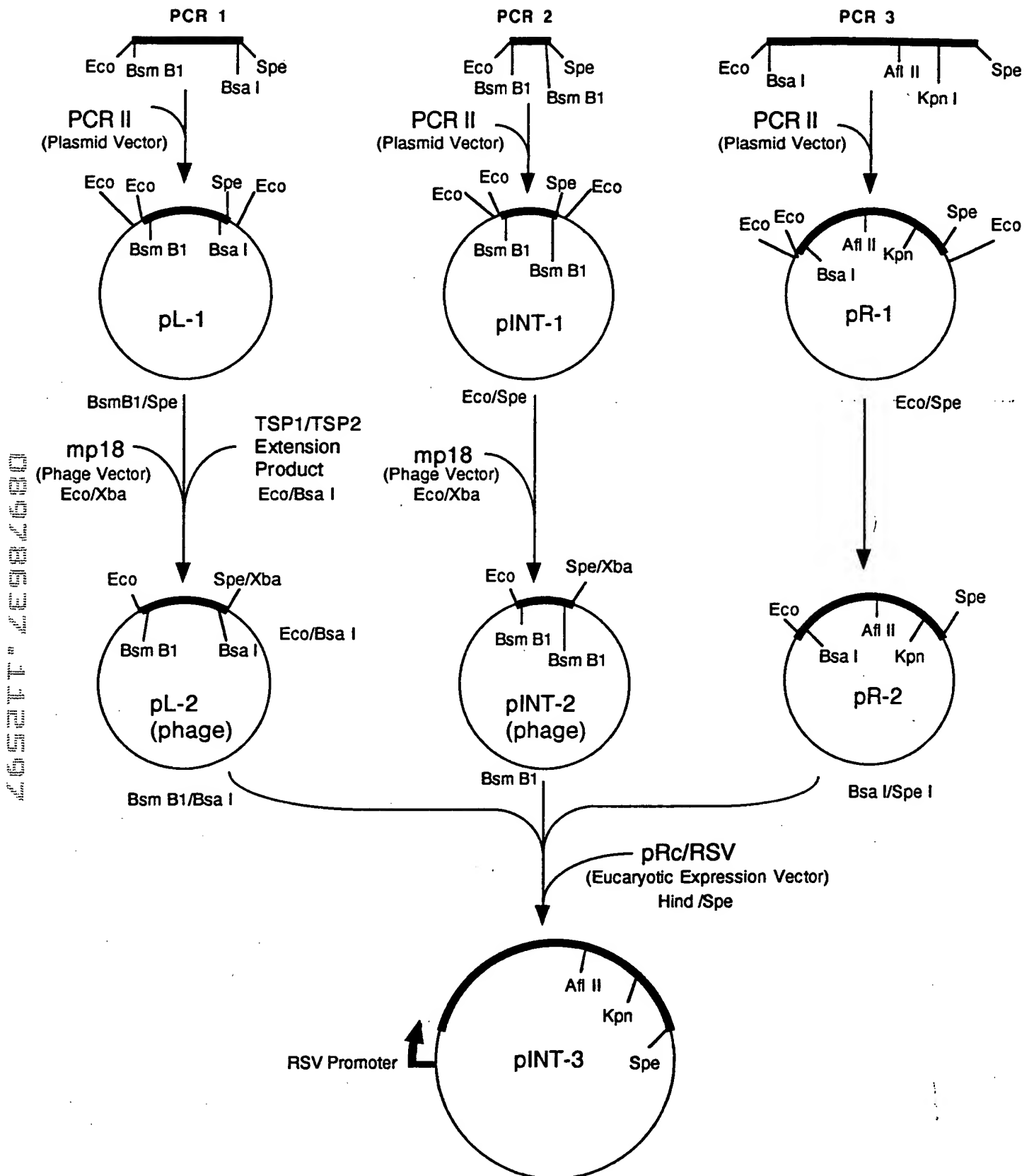


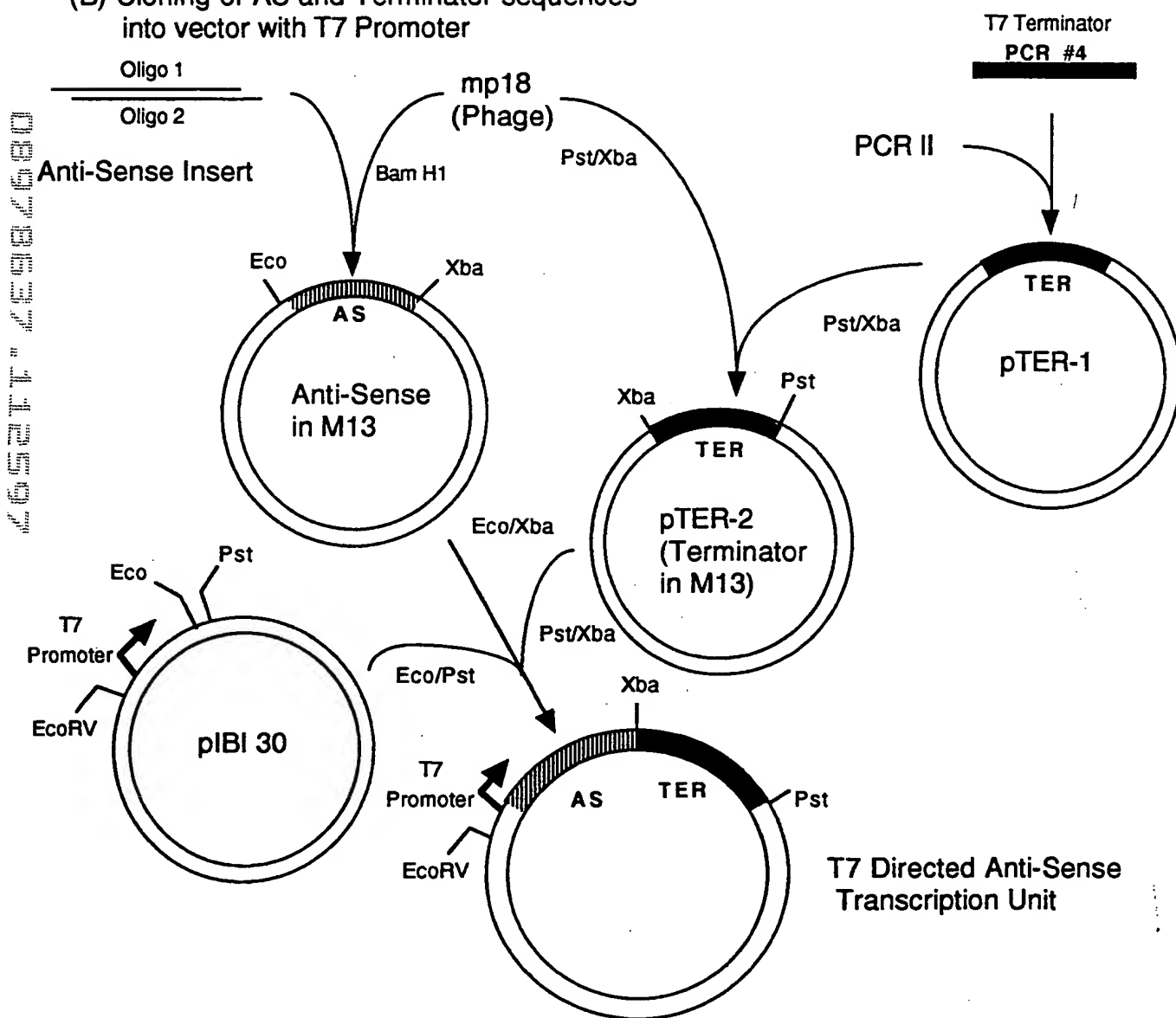
Figure 29

Fusion of PCR Pieces to Construct  
T7 RNA Polymerase with an Intron

## (A) Oligomers

HTA-1	GAT CAT TAG ACC AGA TCT GAG CCT GGG AGC TCT CTG GCT AAC TAG GGA ACC CAC TGCTTA AGC CTC AAG
HTA-2	GAT CCT TGA GGC TTA AGC AGT GGG TTC CCT AGT TAG CCA GAG AGC TCC CAG GCT CAG ATC TGG TCT AAT
HTB-1	GAT CAC CTT AGG CTC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA G
HTB-2	GAT CCT TGA GGA GGT CTT CGT CGC TGT CTC CGC TTC TTC CTG CCA TAG GAG AGC CTA AGG T
HTC-1	GAT CAT AGT GAA TAG AGT TAG GCA GGG ATA CTC ACC ATT ATC GTT TCA GAC CCA CCT CCC AG
HTC-2	GAT CCT GGG AGG TGG GTC TGA AAC GAT AAT GGT GAG TAT CCC TGC CTA ACT CTA TTC ACT AT
TER-1	AAT CTA GAG CTA ACA AAG CCC GAA AGG AAG
TER-2	TTC TGC AGA TAT AGT TCC TCC TTT CAG C

## (B) Cloning of AS and Terminator sequences into vector with T7 Promoter



**Figure 30**  
Insertion of Anti-Sense Sequences into  
T7 Directed Transcription Units

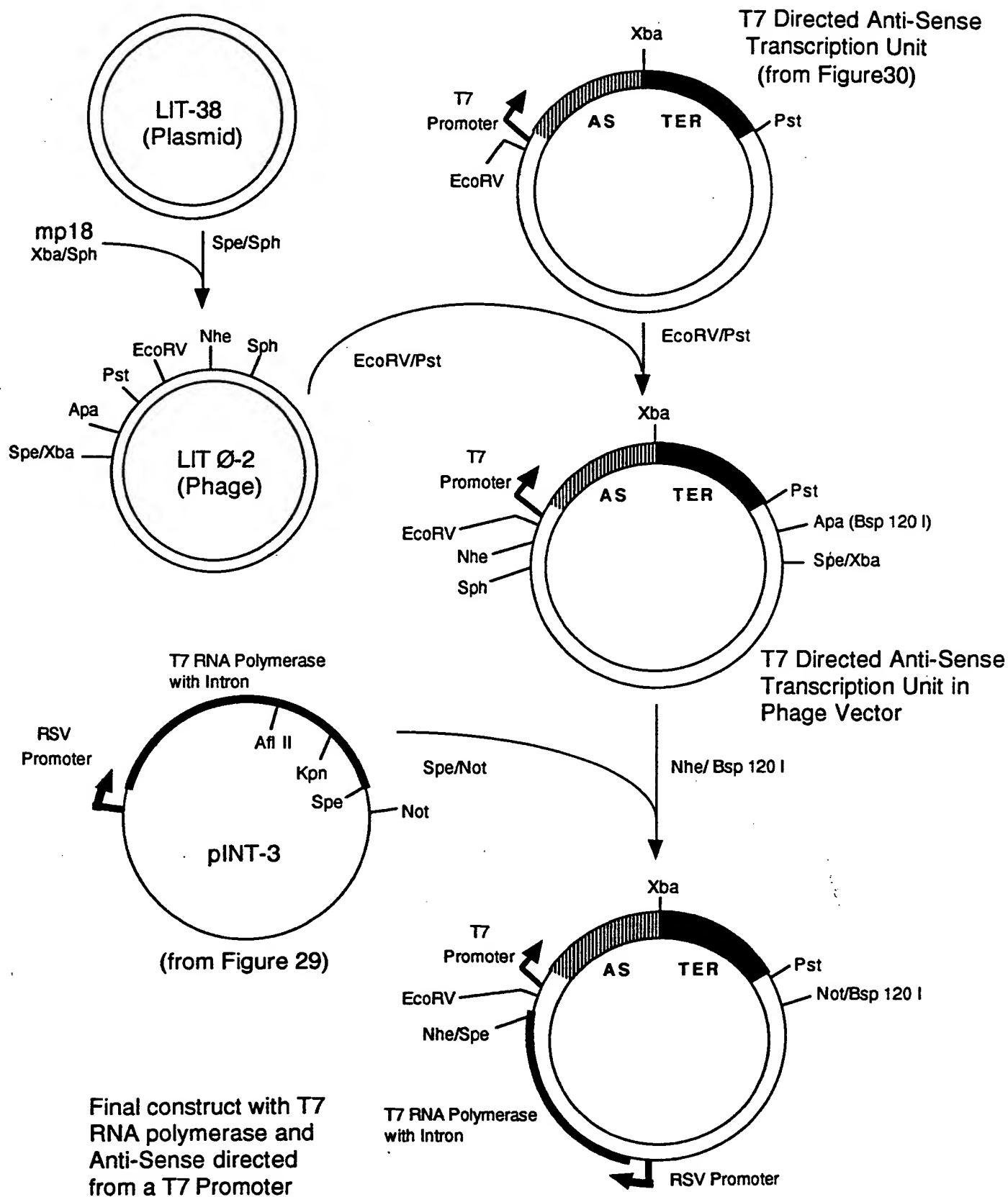


Figure 31

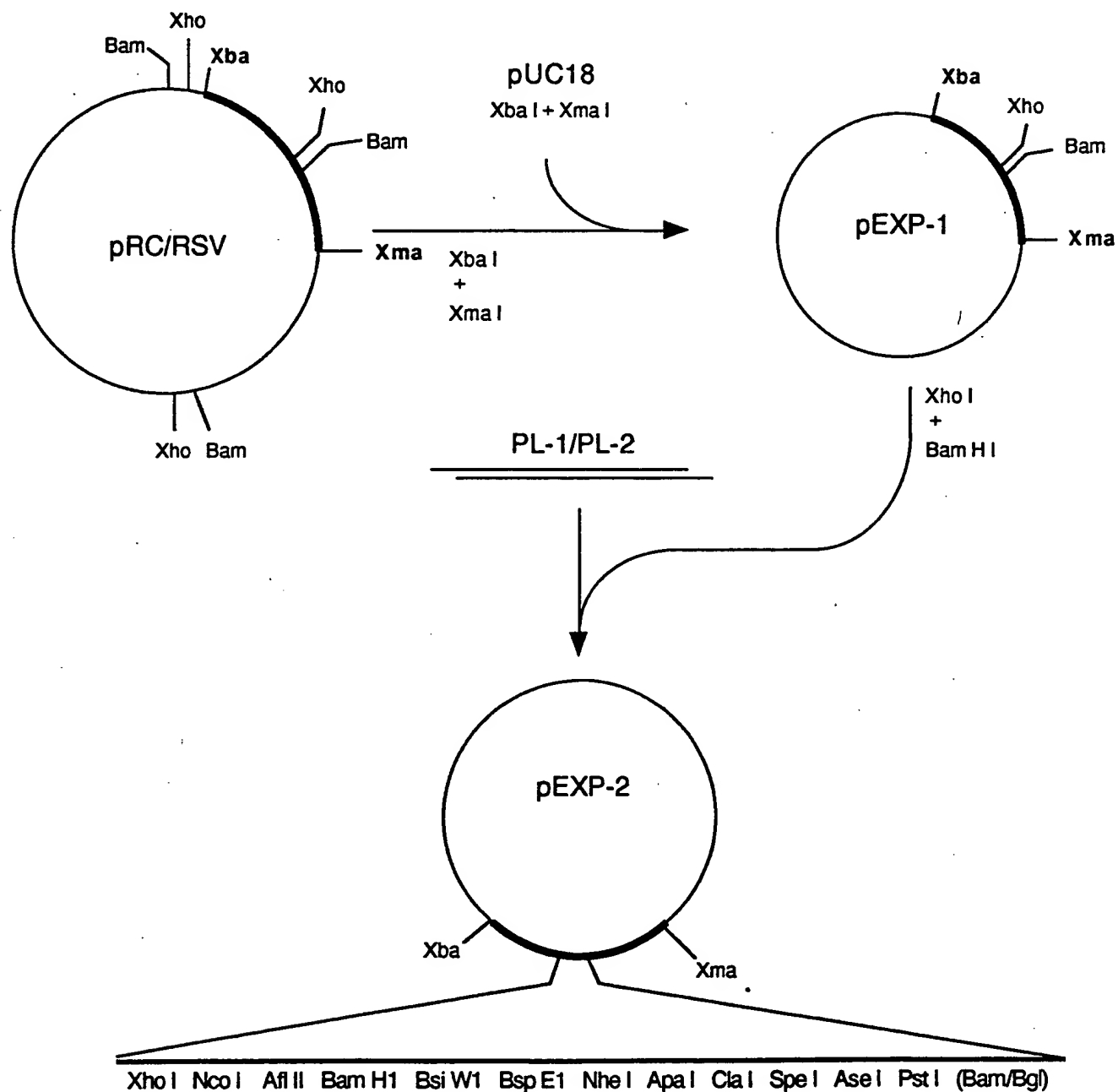
Construct with T7 RNA polymerase and Anti-Sense directed from a T7 Promoter

06978637 1133

## A) Oligomers for introduction of T7 signals and polylinker

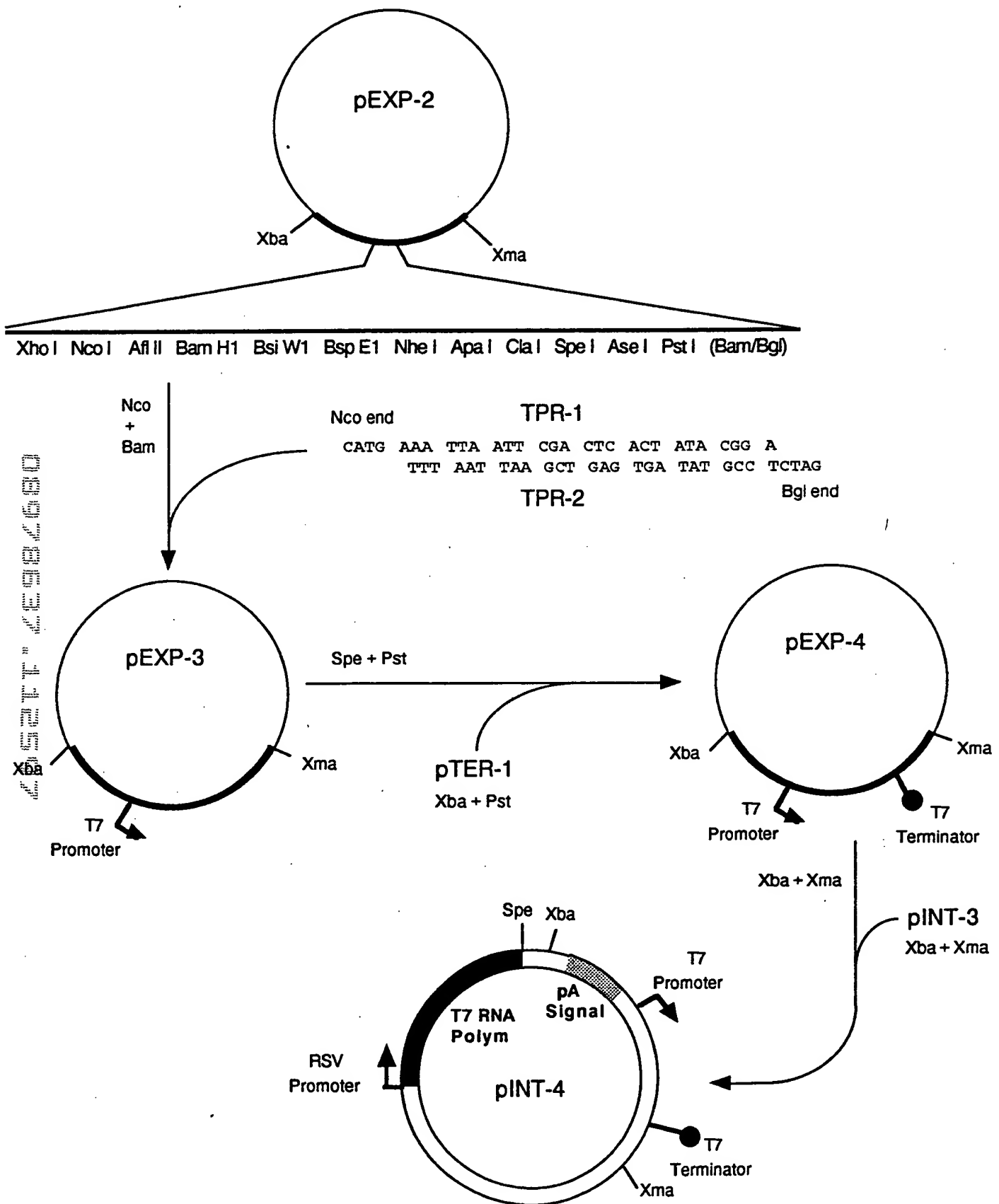
**PL-1** TCG AGC CAT GGC TTA AGG ATC CGT ACG TCC GGA GCT AGC GGG CCC ATC GAT ACT  
AGT TAA ATG CAG ATC T

**PL-2** CTA GAG ATC TGC ATT TAA CTA GTA TCG ATG GGC CCG CTA GCT CCG GAC GTA CGG  
ATC CTT AAG CCA TGG C

**Figure 32**

Introduction of Poly-Linker for Creation of Protein Expression Vector



**Figure 33**

Final steps for construction of Expression Vector

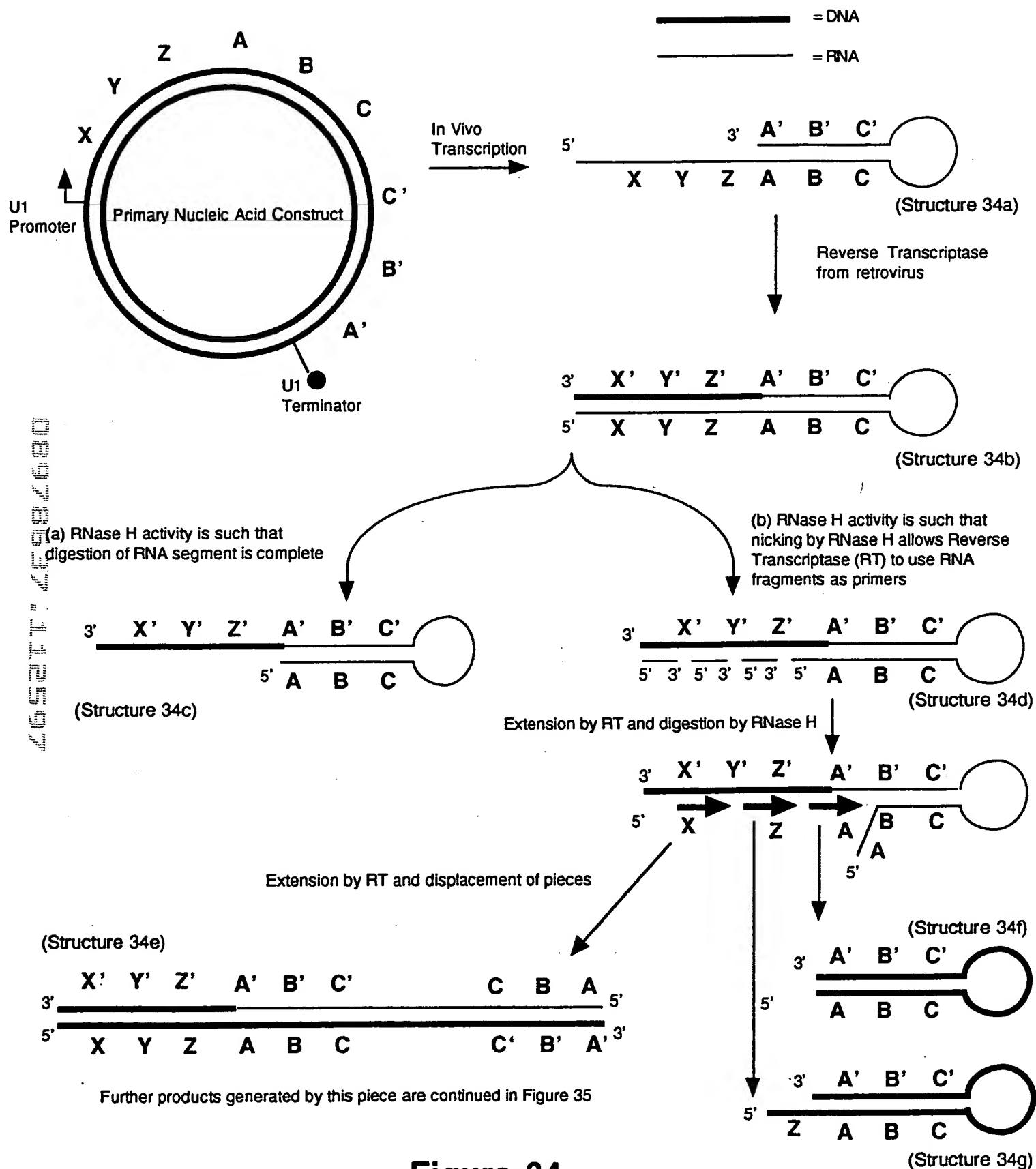
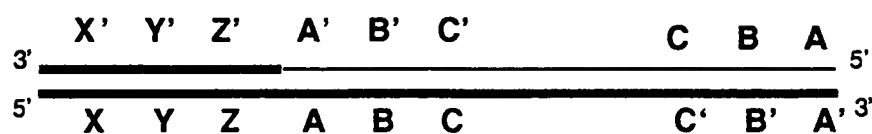


Figure 34

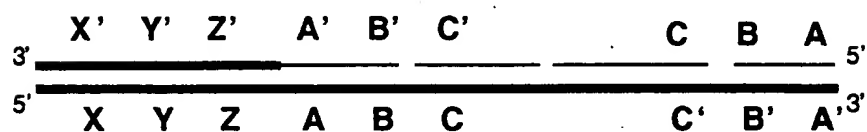
Construct that produces single-stranded Anti-Sense DNA

Continued from Figure 34

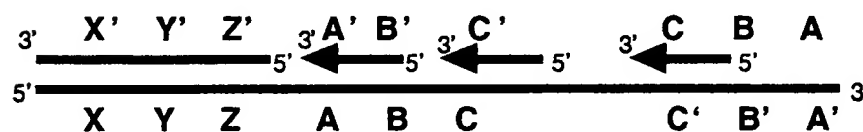
(Structure 34e)



Nicking by RNase H



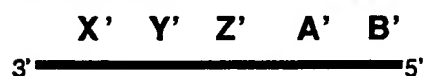
Extension by RT and digestion by RNase H



(Structure 35h)



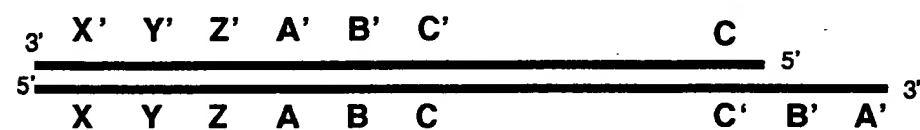
(Structure 35i)



(Structure 35j)

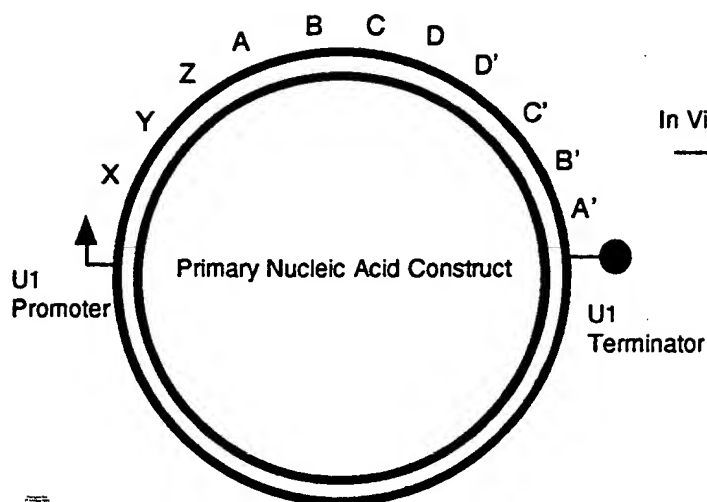


(Structure 35k)

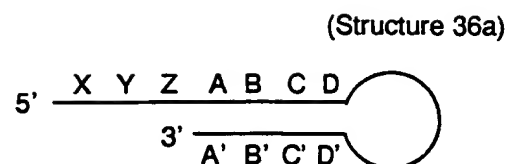


Extension by RT and displacement generates Single-Stranded DNA and a mostly Double-stranded DNA molecule

**Figure 35**  
Continuation of Process from Figure 34



In Vivo Transcription

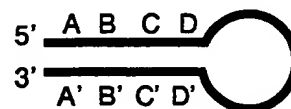


In a series of events similar to that shown for Example G-1, the net products of Rnase H and RT activities on the transcript above create Double-stranded DNA products similar to these below:

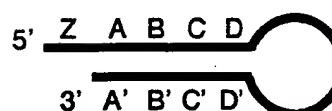
———— = DNA  
 ———— = RNA

In this example, A B C is a promoter sequence, directing transcription off of these Double-stranded DNA products to create RNA transcripts with varying amounts of double-stranded character. Furthermore, the single-stranded loop segment (D to D') of the transcript codes for anti-sense sequences

(Structure 36b)



+ (Structure 36c)

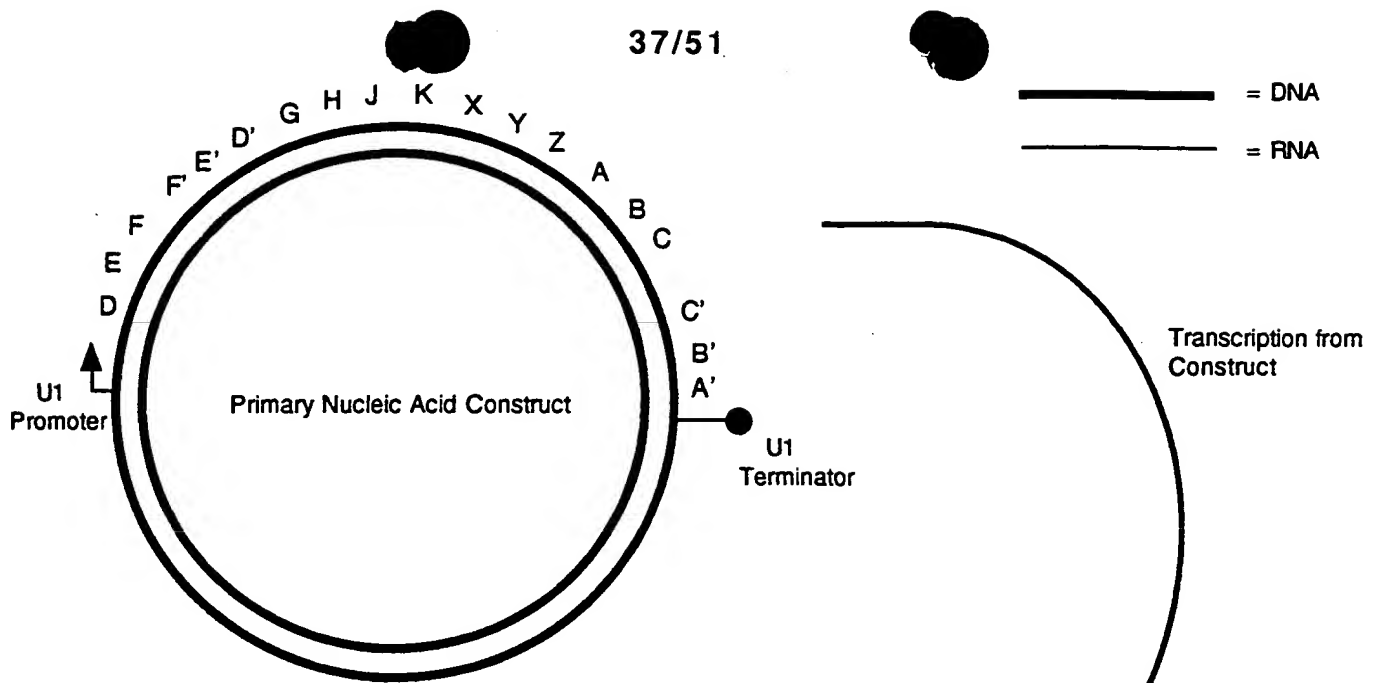


+ (Structure 36d)

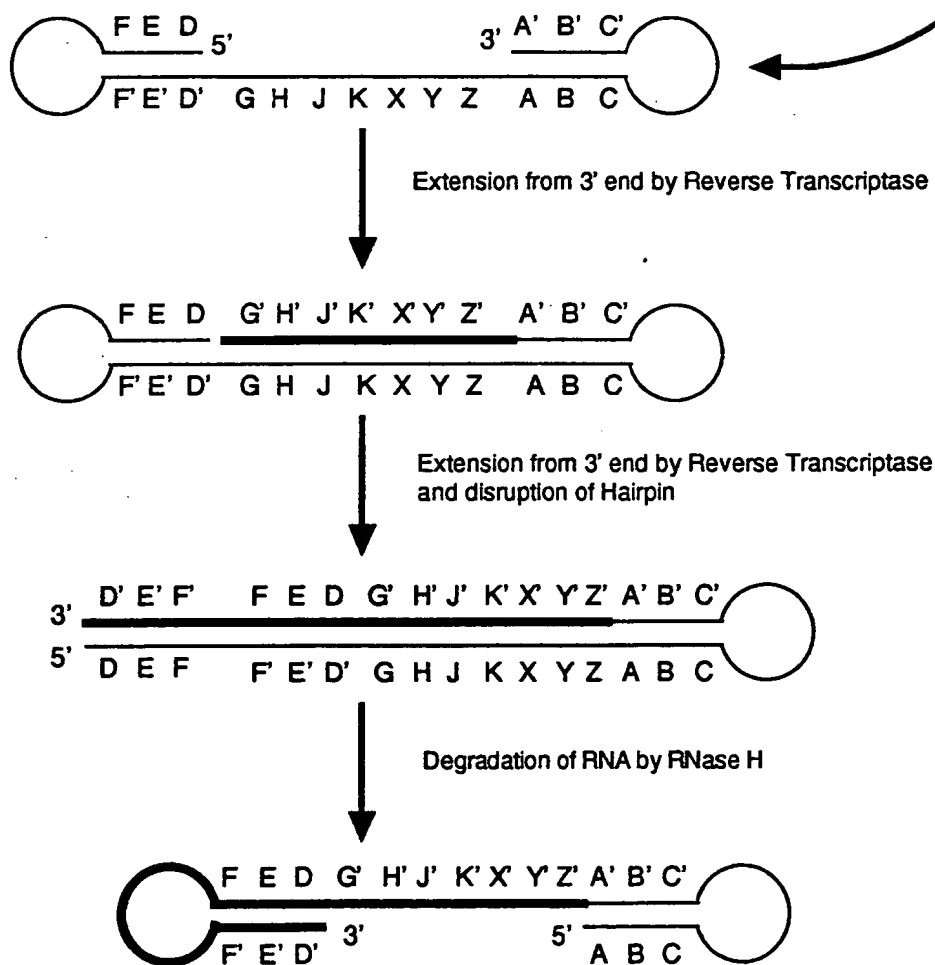


# Figure 36

Construct that produces RNA that is Reverse Transcribed to create Secondary DNA Constructs capable of directing transcription



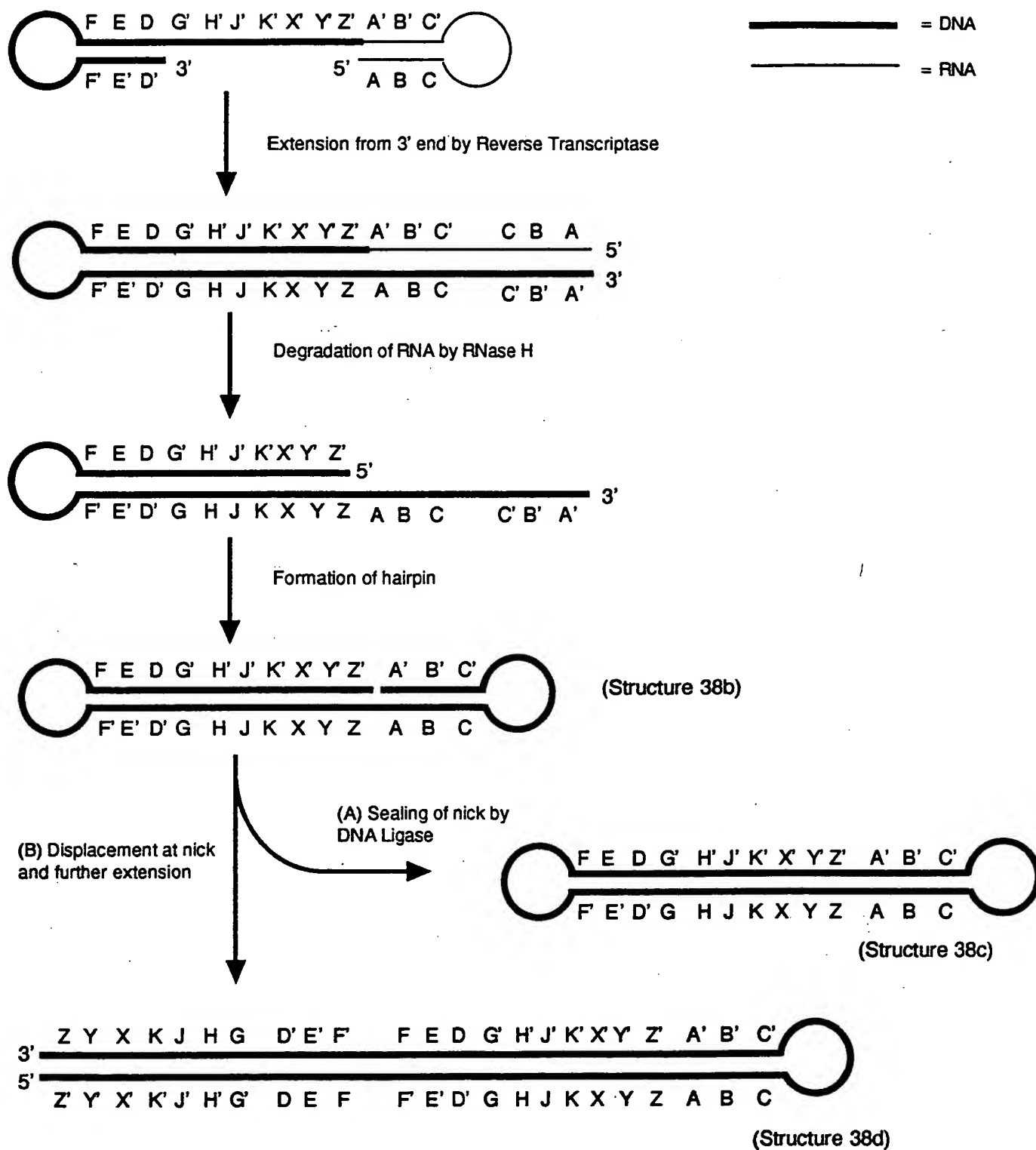
(Structure 37a)



(Continued in Figure 38)

**Figure 37**

Construct which Propagates a Double Hairpin Production Center



**Figure 38**

Continuation of process from Figure 37

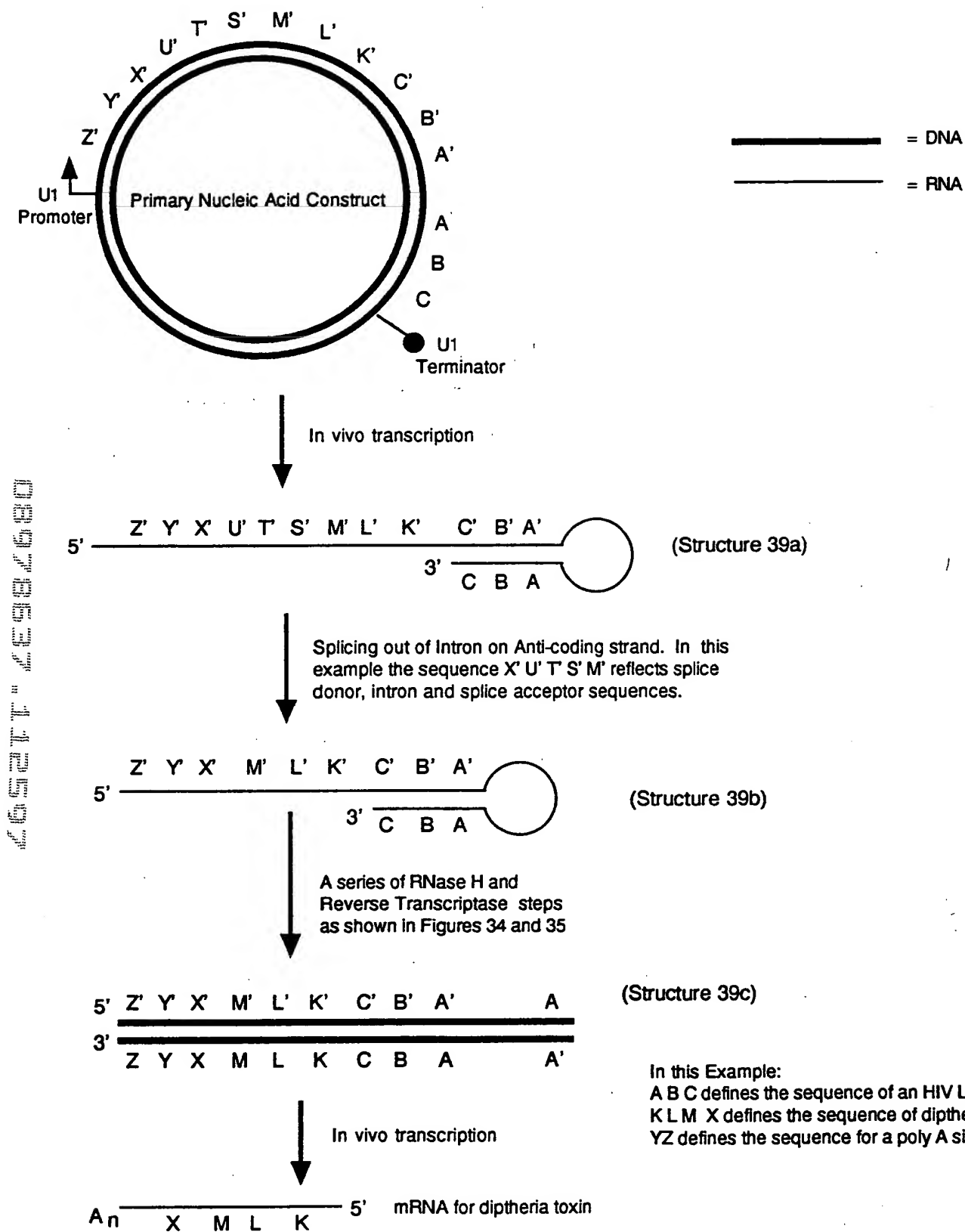
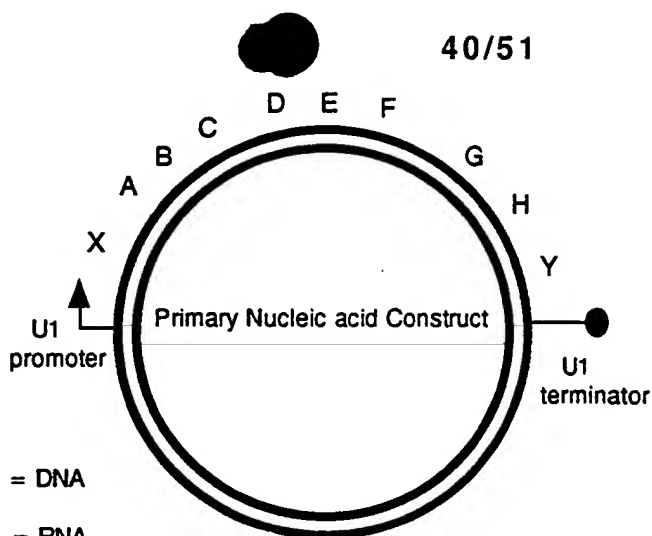


Figure 39

Construct which propagates a Production Center capable of Inducible Suicide



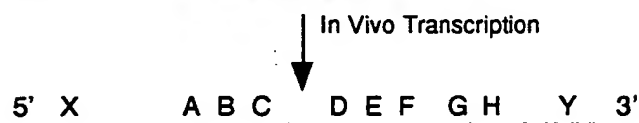
The sequence A B C defines a promoter

The sequence D E F defines an Anti-Sense sequence

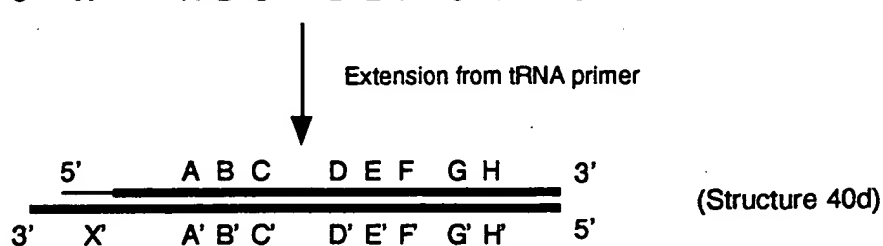
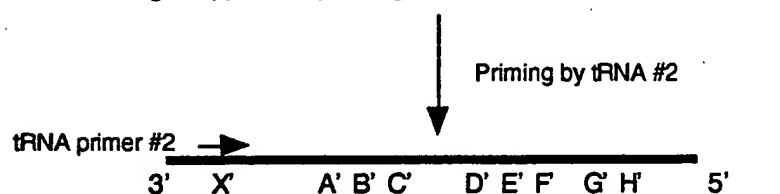
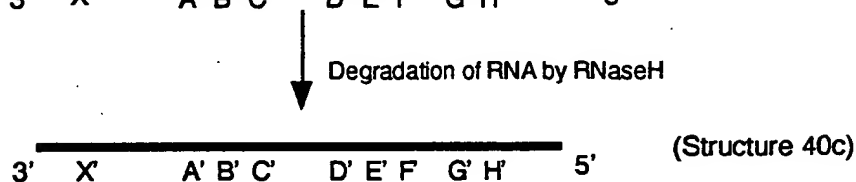
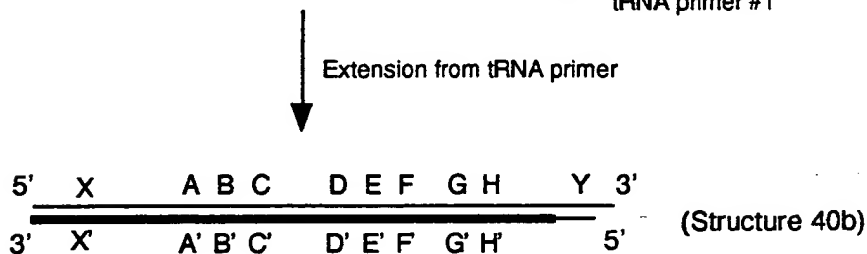
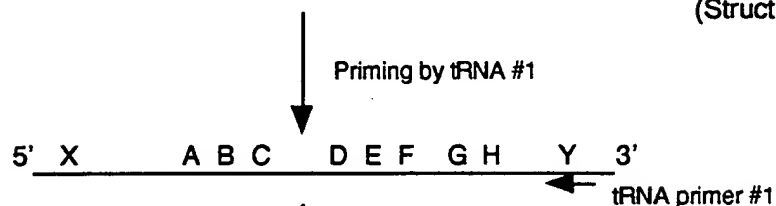
The sequence G H defines a poly A addition site

The sequence defined by Y defines a primer binding site for tRNA primer #1

The sequence defined by X' defines a primer binding site for tRNA primer #2



(Structure 40a)

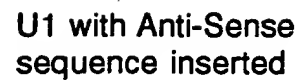
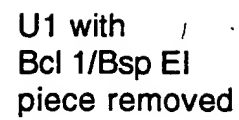


**Figure 40**

Use of tRNA primers to create a DNA construct for secondary production of transcripts

08978637 112597





## Excision of Sequences from U1 Transcript Region and Replacement with Novel Sequences

## (A) Anti-sense oligomers

HVA-1 GAT CCG GAT TGA GGC TTA AGC AGT GGG TTC CCT AGT TAG CCA GAG AGC TCC CAG GCT CAG ATC TGG TCT AAT

HVA-2 CCG GAT TAG ACC AGA TCT GAG CCT GGG AGC TCT CTG GCT AAC TAG GGA ACC CAC TGC TTA AGC CTC AAT CCG

HVB-1 GAT CCG GAC CTT GAG GAG GTC TTC GTC GCT GTC TCC GCT TCT TCC TGC CAT AGG AGA GCC TAA GGT

HVB-2 CCG GAC CTT AGG CTC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA GGT CCG

HVC-1 GAT CCG GAT GGG AGG TGG GTC TGA AAC GAT AAT GGT GAG TAT CCC TGC CTA ACT CTA TTC ACT AT

HVC-2 CCG GAT AGT GAA TAG AGT TAG GCA GGG ATA CTC ACC ATT ATC GTT TCA GAC CCA CCT CCC ATC CG

HVD-1 GAT CAG CAT GCC TGC AGG TCG ACT CTA GAC CCG GGT ACC GAG CTC GCC CTA TAG TGA GT C GTA TTA T

HVD-2 CCG GAT AAT ACG ACT CAC TAT AGG GCG AGC TCG GTA CCC GGG TCT AGA GTC GAC CTG CAG GCA TGC T

## (B) Replacement of U1 sequences with HIV Anti-sense sequences

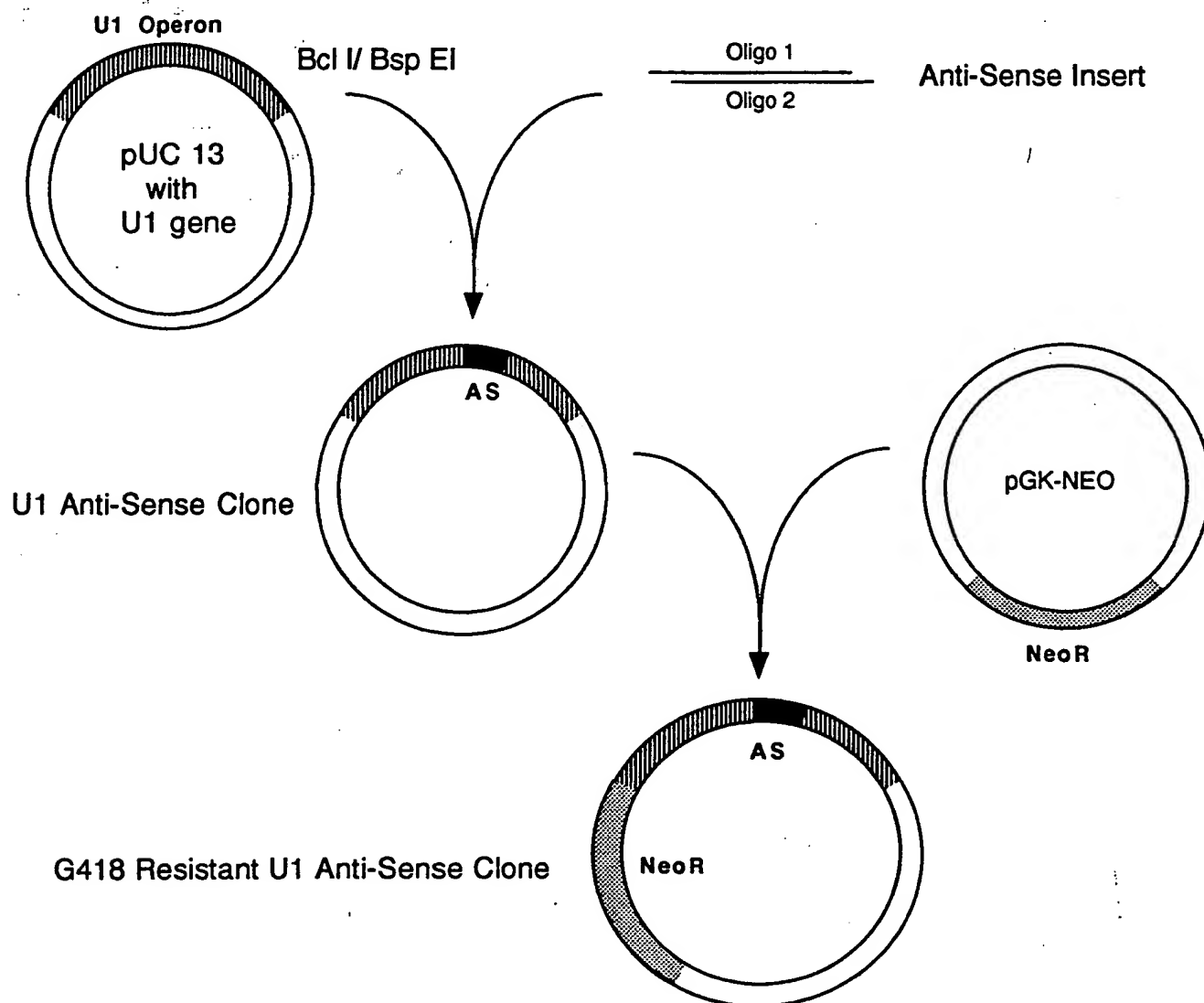
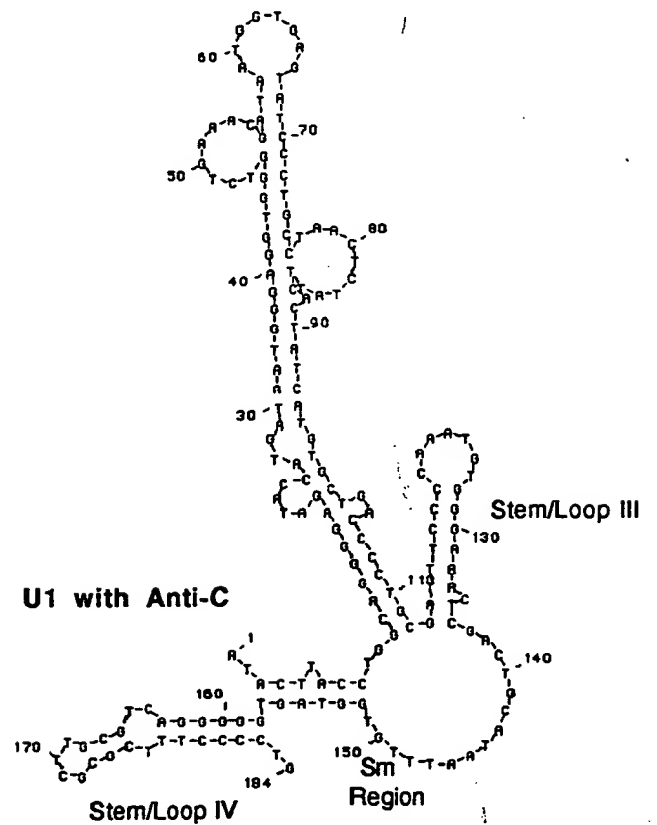
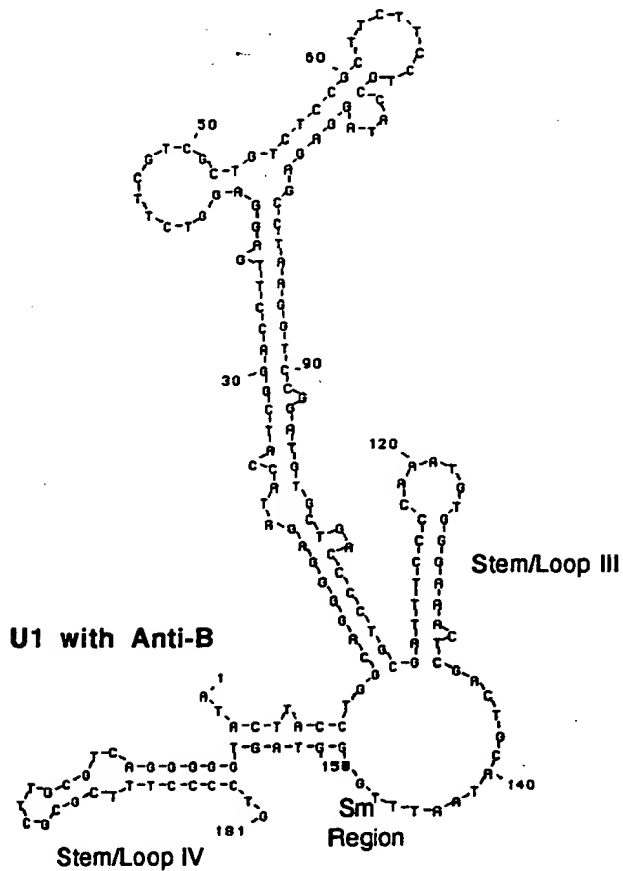
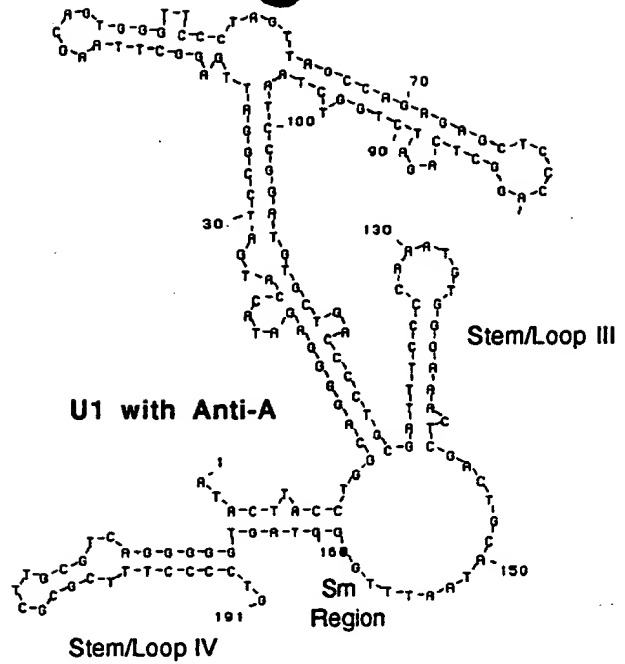
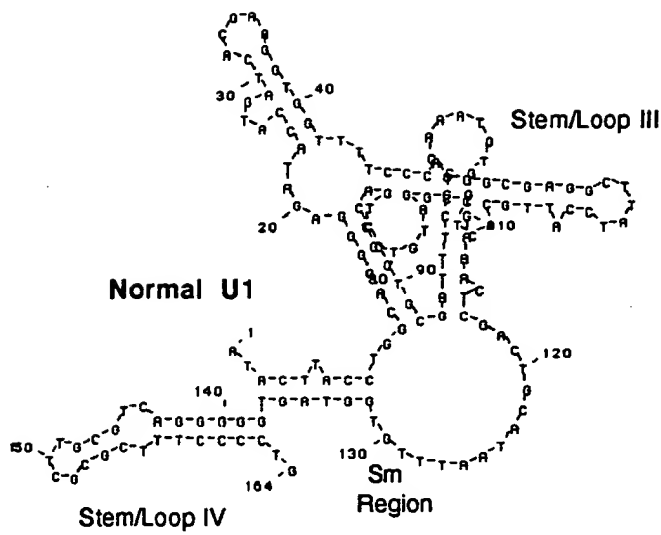


Figure 42

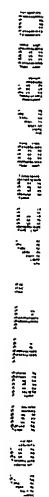
Insertion of Anti-Sense Sequences into U1 Operons



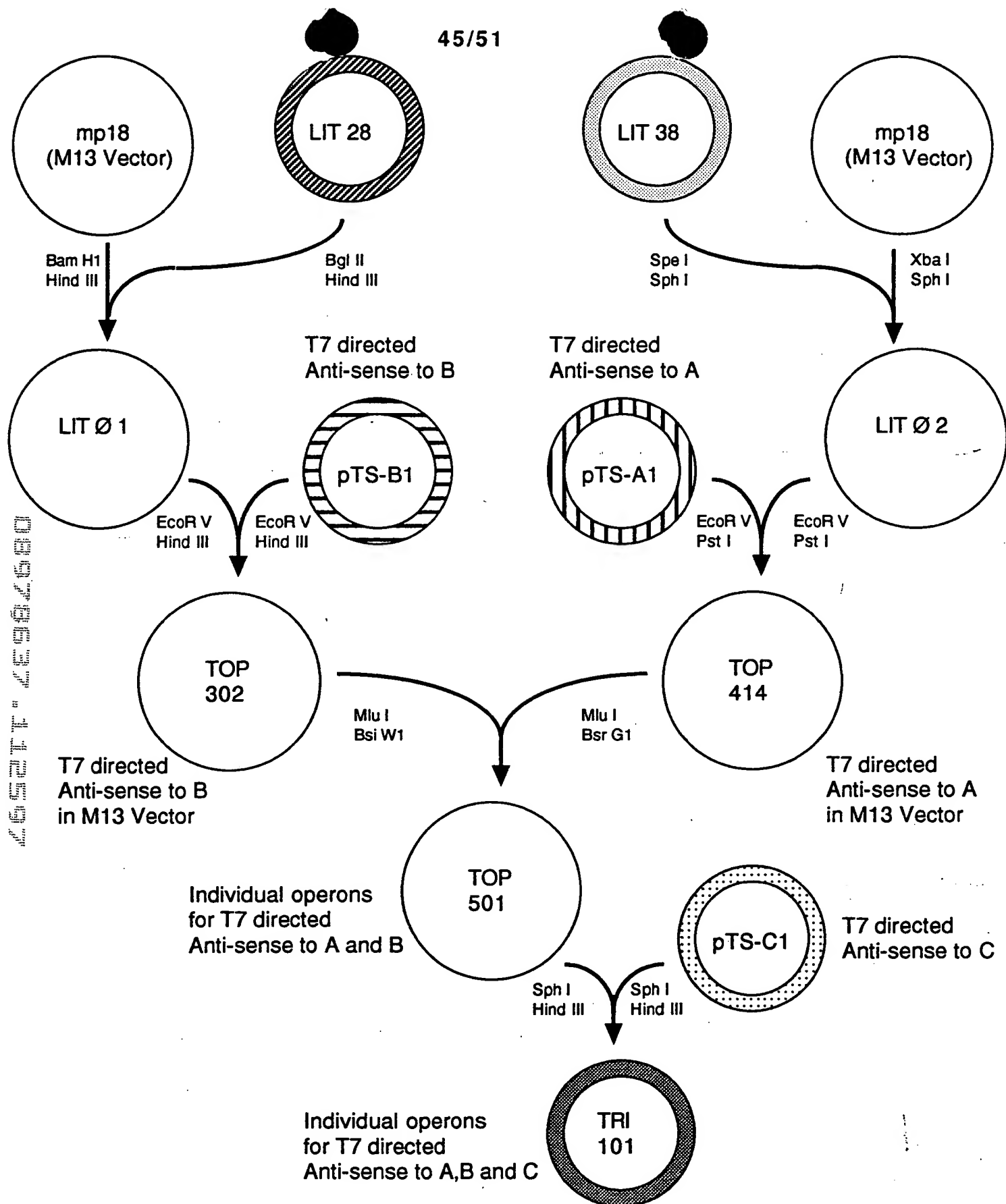
**Figure 43**

Predicted Secondary structures for U1  
Transcripts with Anti-sense Substitutions

00070637 11297



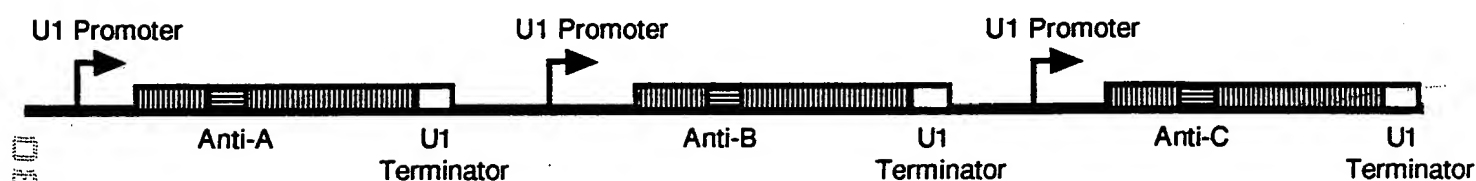
**F** **E** **C** **D**



**Figure 45**  
Construction of T7 Triple Operon

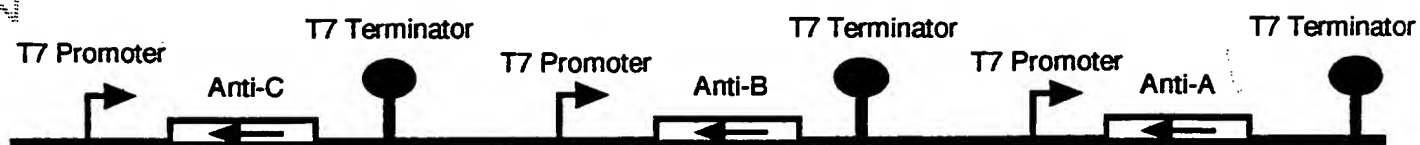
## pNDU1(A,B,C)

Triple U1 Operon Construct with HIV Anti-Sense



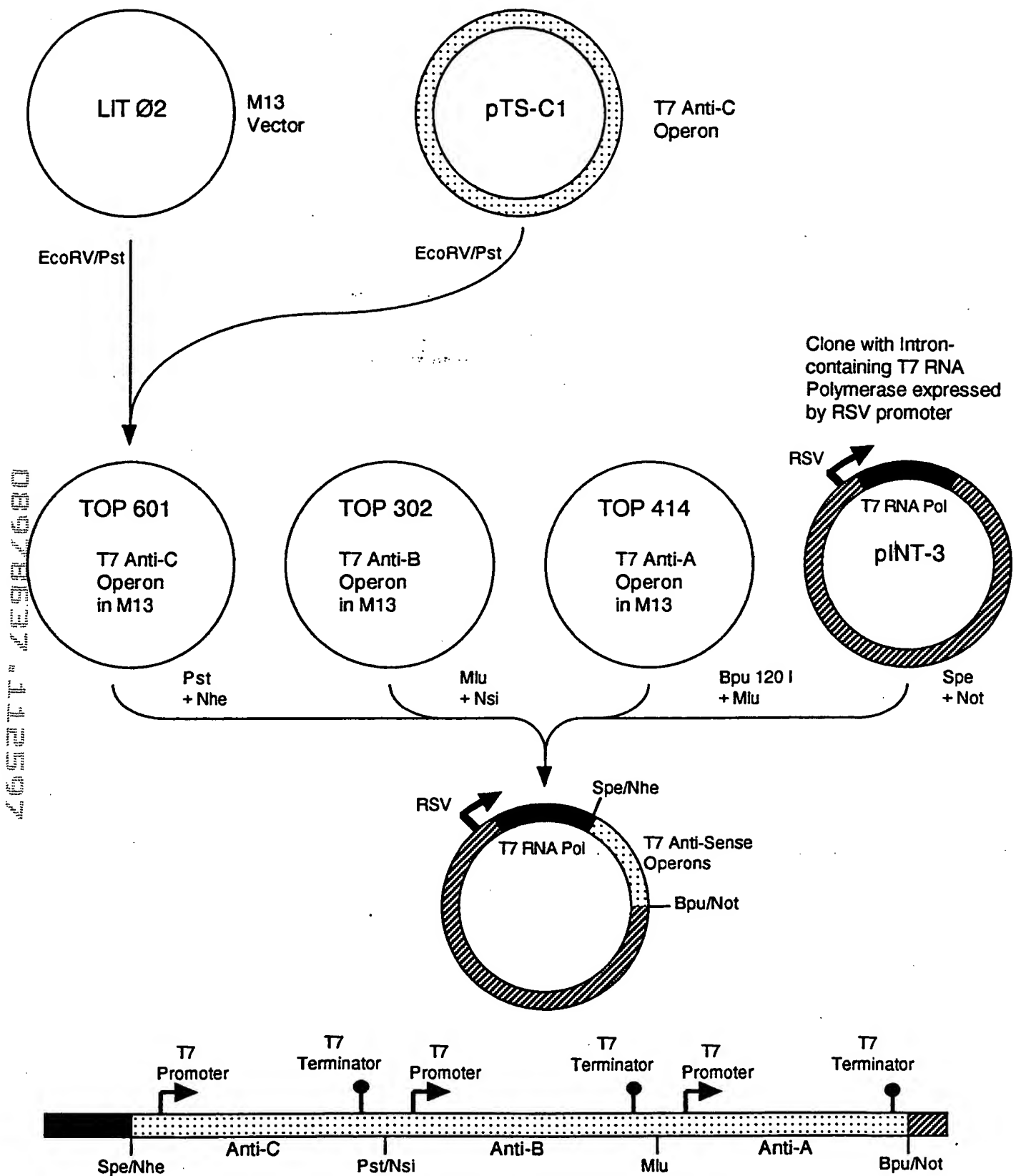
## TRI 101

Triple T7 Operon Construct with HIV Anti-Sense



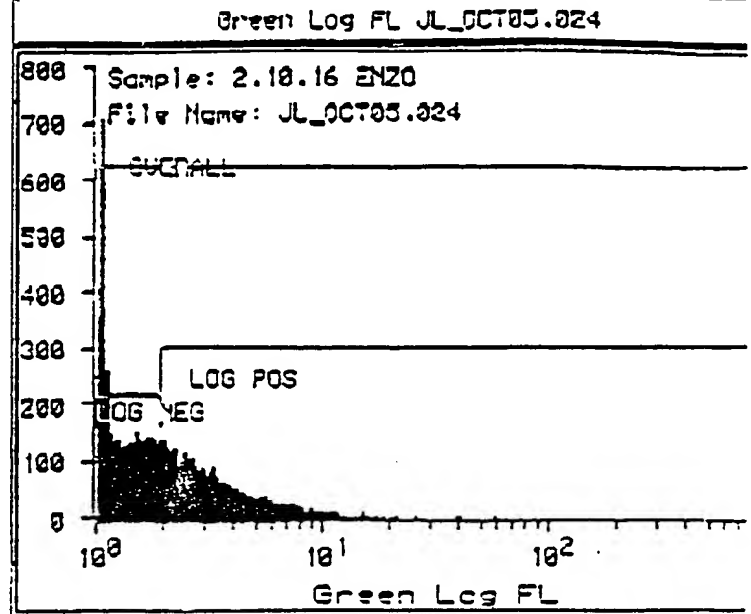
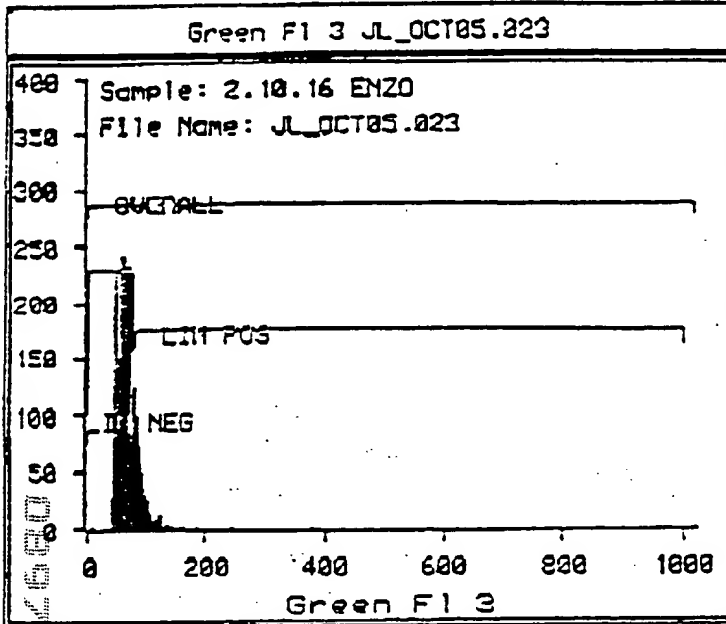
**Figure 46**

Structures of Triple Operon Constructs  
from Figures 44 and 45



**Figure 47**

Construction of Multiple T7 Operons in Vector coding for T7 RNA Polymerase



Global Statistics									
1. Green F1 3 JL_OCT05.023				Total = 7509					
2. Green Log FL JL_OCT05.024				Total = 7509					
Hist	Region	Bounds	Counts	%	Mean X	Mean Y	Mode	xc	
1.	LIN NEG	1 78	5714	76.1	63.65		70	14	
	LIN POS	85 1002	1129	15.0	97.34		85	17	
	OVERALL	1 1024	7509	100.0	70.28		70	23	
2.	LOG NEG	2 2	4211	56.1	2.34		2	21	
	LOG POS	2 1001	3407	45.4	4.76		3	69	
	OVERALL	2 1001	7509	100.0	3.43		2	88	

Figure 48

Flow cytometry data measuring binding of  
anti-CD4+ antibody to HIV resistant U037 cells





Figure 49

PCR amplification of gag region  
indicating absence of HIV in  
viral resistant cell line (2.10.16)  
after challenge

08978637-112593

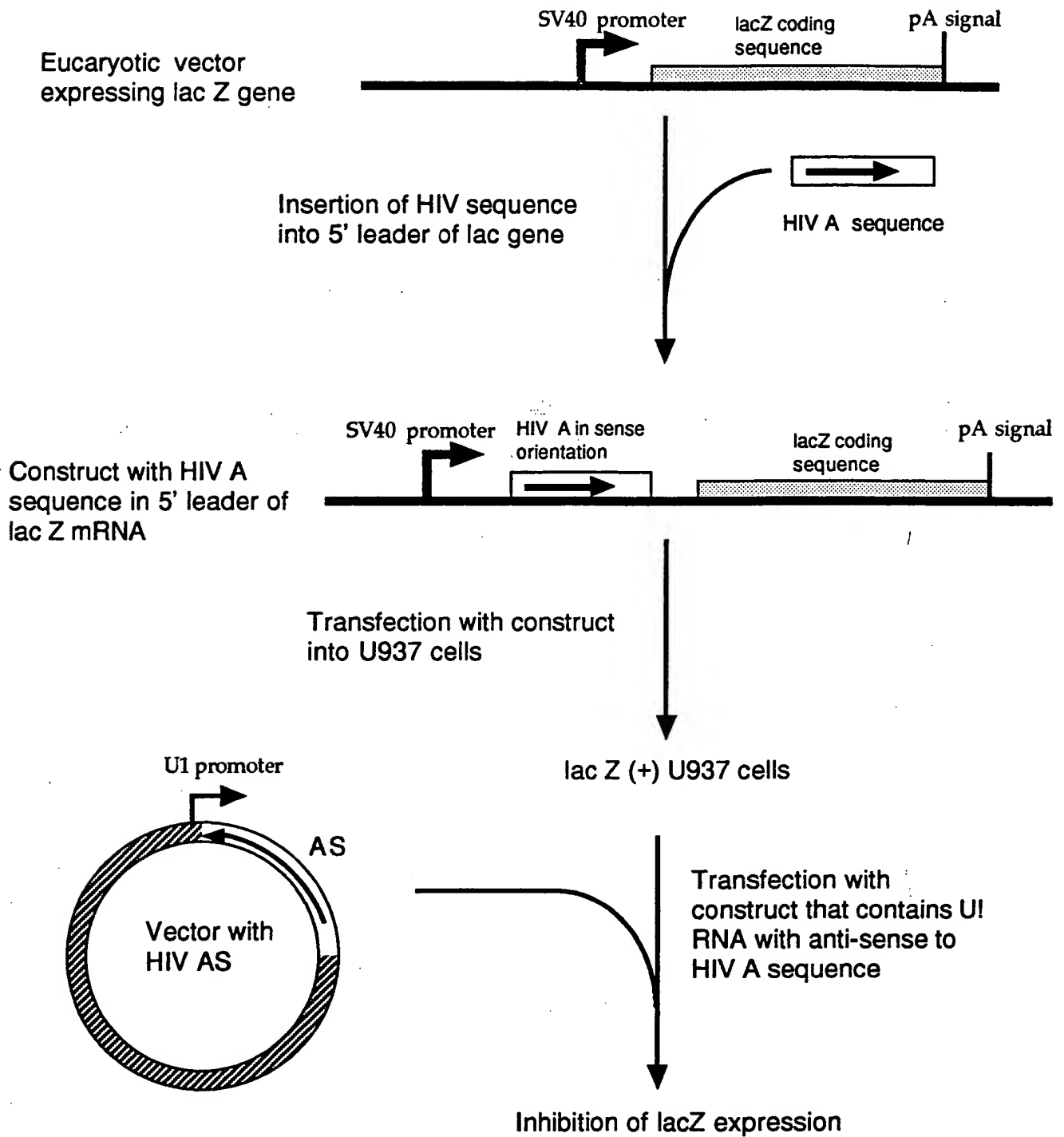


Figure 50

Clone with target-lacZ fusion will have reduced expression of lacZ after transfection by HIV Anti-sense construct

Enzyme activity as expressed by  $A_{420}$  readings  
in extracts prepared from

	$2.5 \times 10^4$ cells	$5 \times 10^4$ cells	$1.0 \times 10^5$ cells
U 937 [untransfected]	0.018	0.023	0.034
U 937 [ HIV A clone ]	0.154	0.277	0.566
U937 [ HIV A / Anti-A]	0.010	0.017	0.027
U 937 [ HIV A/Anti-ABC]	0.013	0.021	0.035
U 937 [ HIV A / Null DNA]	0.120	0.212	0.337

[ B] Expression of Beta-galactosidase activity by *In situ* assay :

U 937 [ untransfected ] no blue spots in cells

U 937 [ HIV A clone ] blue spots in cells

U 937 [ HIV A/Anti A ] no blue spots in cells

U 937 [ HIV A/Anti ABC] no blue spots in cells

U 937 [ HIV A / Null DNA] blue spots in cells

Figure 51

Expression of Beta-galactosidase activity  
in extracts

265217 46982580